VOLUME 32 JULY 1954 NUMBER 4

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF BOTANY

(Formerly Section C, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues annually THE CANADIAN JOURNAL OF BOTANY and six other journals devoted to the publication of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; the members representing the Royal Society of Canada and four other scientific societies.

EDITORIAL BOARD

Representatives of the National Research Council

A. N. CAMPBELL, *University of Manitoba*
G. E. HALL, *University of Western Ontario*
E. G. D. MURRAY, *McGill University*
D. L. THOMSON, *McGill University*
W. H. WATSON (Chairman), *University of Toronto*

Editors of the Journals

D. L. BAILEY, *University of Toronto*
J. B. COLLIP, *University of Western Ontario*
E. H. CRAIGIE, *University of Toronto*
G. A. LEDINGHAM, *National Research Council*
LÉO MARION, *National Research Council*
R. G. E. MURRAY, *University of Western Ontario*
G. M. VOLKOFF, *University of British Columbia*

Representatives of Societies

D. L. BAILEY, *University of Toronto*
Royal Society of Canada
J. B. COLLIP, *University of Western Ontario*
Canadian Physiological Society
E. H. CRAIGIE, *University of Toronto*
Royal Society of Canada
R. G. E. MURRAY, *University of Western Ontario*
Canadian Society of Microbiologists
H. G. THODE, *McMaster University*
Chemical Institute of Canada
T. THORVALDSON, *University of Saskatchewan*
Royal Society of Canada
G. M. VOLKOFF, *University of British Columbia*
Royal Society of Canada; Canadian Association of Physicists

Ex officio

LÉO MARION (Editor-in-Chief), *National Research Council*

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Botany, National Research Council, Ottawa 2, Canada.

(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover))

Proof, correspondence concerning proof, and *orders for reprints* should be sent to the Manager, Editorial Office (Research Journals), Division of Administration, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, and orders for single or back numbers should be sent to Division of Administration, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Bimonthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00
Canadian Journal of Chemistry	Monthly	$5.00
Canadian Journal of Microbiology*	Bimonthly	$3.00
Canadian Journal of Physics	Monthly	$4.00
Canadian Journal of Technology	Bimonthly	$3.00
Canadian Journal of Zoology	Bimonthly	$3.00

The price of single numbers of all journals is 75 cents.

** Volume 1 will combine three numbers published in 1954 with six published in 1955 and will be available at the regular annual subscription rate of $3.00.*

Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 32 JULY 1954 NUMBER 4

DETECTION OF VITAMIN B_{12} PRODUCTION BY MICROORGANISMS USING *EUGLENA GRACILIS* VAR. *BACILLARIS* AS THE ASSAY ORGANISM[1]

BY E. R. WITKUS, F. GENTILE, L. KELLY, AND L. MURPHY

Abstract

Using *Euglena gracilis* var. *bacillaris* as the assay organism a study was made of the ability of 21 different bacteria to synthesize vitamin B_{12}. Thirteen of the 21 organisms studied were found to secrete vitamin B_{12}.

Euglena gracilis var. *bacillaris* was found to exhibit a quantitative growth response to vitamin B_{12} (1). Robbins, Hervey, and Stebbins (2 and 3) employed *Euglena gracilis* var. *bacillaris* as an assay organism for vitamin B_{12} detection. These authors reported that a large number of bacteria, particularly the actinomycetes, can synthesize substantial amounts of vitamin B_{12}, whereas the yeasts and filamentous fungi can synthesize only small quantities of this vitamin. Using *Euglena gracilis* var. *bacillaris* as the assay organism an attempt was made to determine which of the common bacteria synthesize vitamin B_{12}.

Experimental

Euglena gracilis var. *bacillaris* was grown in the light in nutrient broth (pH 6.8) at 28°–30° C. The organisms tested for their vitamin B_{12} activity were: *Aerobacter aerogenes*, *Bacillus cereus*, *B. megatherium*, *B. subtilis*, *Corynebacterium xerose*, *Escherichia coli*, *Micrococcus roseus*, *Mycobacterium lacticola*, *M. smegmatis*, *Proteus vulgaris*, *Sarcina flava*, *S. lutea*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptomyces aureofaciens*, *S. fradiae*, *S. griseus*, *S. lavendulae*, *S. rimosus*, *S. roseochromogenus*, and *S. venezuelae*.

In determining whether these bacteria secreted vitamin B_{12}, the agar streak method was used. Ten cubic centimeters of vitamin B_{12} deficient agar (pH 6.8) inoculated with 1 cc. of a nutrient broth culture (five days old) of *Euglena* was poured into sterile Petri dishes. The agar was allowed to solidify. The surface of the agar in the center of the dish was then streaked with the various organisms previously cultured on vitamin B_{12} deficient agar. The vitamin B_{12} deficient medium used was one suggested by Robbins, Hervey,

[1] *Manuscript received March 3, 1954.*
Contribution from Biological Laboratory, Fordham University, New York, N.Y.

[The May number of this Journal (Can. J. Botany, 32 : 369-502. 1954) was issued June 1, 1954.]

and Stebbins (2) and referred to by them as Medium III. The dish was then inverted and kept in the light at 28° C. for from 10 days to two weeks. This long period of incubation was found to be necessary for good growth of *Euglena*. The dishes were examined for the presence of a zone of stimulation in the vicinity of the streak.

Results

Zones of stimulation of *Euglena* were noted in the case of *Aerobacter aerogenes*, *Bacillus cereus*, *B. megatherium*, *Escherichia coli*, *Micrococcus roseus*, *Mycobacterium lacticola*, *M. smegmatis*, *Proteus vulgaris*, *S. aureofaciens*, *S. fradiae*, *S. griseus*, *S. roseochromogenus*, and *S. venezuelae*. Of the nonpigmented bacteria studied *Bacillus subtilis* and *Corynebacterium xerose* failed to secrete vitamin B_{12} at least in sufficient quantities to stimulate the growth of *Euglena*. *B. subtilis* grew well on the vitamin B_{12} deficient medium, while *C. xerose* grew very poorly. *B. megatherium* produced the largest zone of stimulation (9 mm.). Of the pigmented organisms studied *Micrococcus roseus* produced the largest zone (6 mm., Fig. 1). *Mycobacterium lacticola* and *M. smegmatis* both produced 4 mm. zones of stimulation. Of the actinomycetes studied, *Streptomyces venezuelae* produced the largest zone of stimulation (12 mm.). In the case of *S. aureofaciens* a 7 mm. zone of stimulation was produced. *S. griseus* and *S. roseochromogenus* both produced 4 mm. zones of stimulation. Of the pigmented organisms, *Sarcina lutea*, *S. flava*, *Serratia marcescens*, and *Staphylococcus aureus* failed to secrete vitamin B_{12}.

It was observed that two of the *Streptomyces* studied inhibited the growth of *Euglena*. *S. lavendulae* produced a zone of inhibition and a zone of stimulation. The zone of stimulation was evident since it was larger than the zone of inhibition. The zone of inhibition was 4 mm. in diameter. Outside of this zone of inhibition there was a dense growth of *Euglena*. In the case of *S. rimosus*, since the zone of inhibition was large (14 mm.), it was impossible to determine whether this particular species does secrete any vitamin B_{12}. It is possible that the vitamin was secreted by the organism, but that the zone of stimulation was smaller than the zone of inhibition.

Summary

1. Using *Euglena gracilis* var. *bacillaris* as the assay organism it was demonstrated that the following bacteria secrete sufficient vitamin B_{12} or its equivalent to stimulate the growth of *Euglena*: *Aerobacter aerogenes*, *Bacillus cereus*, *B. megatherium*, *Escherichia coli*, *Micrococcus roseus*, *Mycobacterium lacticola*, *M. smegmatis*, *Proteus vulgaris*, *Streptomyces aureofaciens*, *S. fradiae*, *S. griseus*, *S. roseochromogenus*, and *S. venezuelae*.

2. Of the nonpigmented bacteria, *Bacillus megatherium* was shown to secrete the most vitamin B_{12}. Of the chromogenic organisms, *Micrococcus roseus* was the most active producer of vitamin B_{12} and of the actinomycetes, *Streptomyces venezuelae* was found to secrete the greatest amount of vitamin B_{12}.

PLATE I

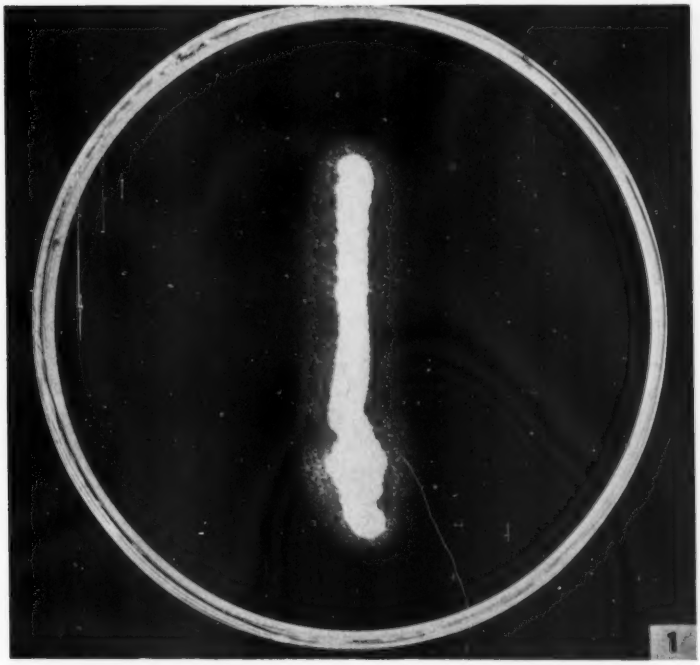


FIG. 1. Photograph showing zone of stimulation of *Euglena gracilis* var. *bacillaris* around streak of *Micrococcus roseus*.

3. Two species of *Streptomyces*, *S. rimosus*, and *S. lavendulae*, inhibited the growth of *Euglena*. *S. lavendulae* produced a zone of stimulation that apparently was larger than the zone of inhibition. In the case of *S. rimosus*, no vitamin B_{12} secretion could be detected, because of the large size of the zone of inhibition.

References

1. HUTNER, S. H., PROVASOLI, L., STOCKSTAD, E. L. R., HOFFMAN, C. E., BELL, M., FRANKLIN, A. L., and JUKES, T. H. Assay of the antipernicious anemia factor with *Euglena*. Proc. Soc. Exptl. Biol. Med. 70 : 118-120. 1949.
2. ROBBINS, W. J., HERVEY, A., and STEBBINS, M. E. Studies on *Euglena*. Bull. Torrey Club, 77 : 423-441. 1950*a*.
3. ROBBINS, W. J., HERVEY, A., and STEBBINS, M. E. Studies on *Euglena* and vitamin B_{12} Science, 112 : 455. 1950*b*.

SELFING STUDIES WITH PHYSIOLOGIC RACES OF WHEAT STEM RUST, *PUCCINIA GRAMINIS* VAR. *TRITICI*[1]

By T. Johnson[2]

Abstract

Forty-two cultures of wheat stem rust (*Puccinia graminis* var. *tritici* Erikss. and Henn.), comprising 34 physiologic races, were subjected to selfing studies each of which involved the inoculation of barberry plants with the sporidia of a race, and the determination of the races in the uredial cultures derived from the aeciospores. In all of the cultures studied, the pathogenic properties expressed on the differential hosts appeared to be inherited according to the same principles. On the varieties Marquis and Kota (*Triticum vulgare*), pathogenicity of the races in the progeny tended to resemble that of the parent race. On Reliance (*T. vulgare*), avirulence was a dominant character, virulence a recessive one. On the durum wheats Arnautka, Mindum, and Spelmar, virulence was a dominant and avirulence a recessive character. On Einkorn (*T. monococcum*) and on Vernal (*T. dicoccum*), avirulence was dominant to virulence. In the progenies of some races, pathogenic variation occurred though it did not transgress the circumscribed limits of the parent race; the population therefore consisted chiefly of substrains (*biotypes*) of the same race.

Introduction

The frequent appearance of new races and subraces (biotypes) in stem rust of wheat (*Puccinia graminis* var. *tritici* Erikss. & Henn.) raises the question of how these races originate. One known source of new races is "hybridization" i.e. the "crossing" and "selfing" of races. The two are not essentially different. Races are apparently quite interfertile so that new associations of their genetic elements can occur readily, a process referred to as "crossing" for want of a better term. "Selfing" is essentially the same process, connoting the reassociation of the genetic elements within a race.

It has been the practice of the writer for many years to inoculate plants of barberry (*Berberis vulgaris* L.) with sporidia of known races that had been carefully checked for purity just prior to the formation of the teliospores. In the course of time, a considerable amount of information has accumulated on the inheritance, in selfing studies, of the pathogenic properties of the rust, particularly of those pathogenic properties that are expressed by the infection types formed on the differential hosts. Some attention has also been given to the question of the extent to which known races contained genetical elements that could give rise to rust strains capable of parasitizing some of the rust-resistant wheat varieties now used by plant breeders as sources of stem-rust resistance. In all the work, there was a basic assumption that a broadly based study of the inheritance of the pathogenic characters of physiologic races would increase knowledge to such a degree that it would be possible to make predictions from the phenotype of a race as to the pathogenic character of its progeny. Though this aim has not been fully realized it is now evident

[1] *Manuscript received February 17, 1954.*
Contribution No. 1369 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] *Officer-in-Charge. Plant Pathology Laboratory, Winnipeg, Man.*

that the inheritance of some of the characteristics expressed on the differential hosts follows well-defined rules, the knowledge of which makes possible a forecast of the general pathogenic character of the progeny, though it is rarely possible to predict exactly which races will occur in it.

In the present paper, most of the available data, obtained since about 1935, on the selfing of physiologic races of wheat stem rust have been assembled (Tables I, II, and III) and, as far as possible, interpreted. Some of the earlier work was done in conjunction with Dr. Margaret Newton, the writer's co-worker until 1945. In some of the more recent studies the author had the assistance of Dr. Gordon J. Green.

Materials and Methods

Most of the races subjected to selfing studies were field cultures collected in various places in Canada. The cultures kept for these studies were either initially pure races or were purified as monouredial isolates. Each culture was, therefore, not necessarily a single uredial clone but may have consisted of two or more clones that might be somewhat different genotypically though phenotypically alike at least as far as differential-host reactions are concerned. From the point of view of genetical studies the possible genotypic diversity in a culture may be a disadvantage, but from the standpoint of the present study, which is concerned with the inheritance of the pathogenic properties of races as they occur in nature, it was thought preferable to work with the races in, as nearly as possible, a natural condition.

Teliospores were occasionally produced on seedling leaves but most frequently on the stems of adult plants of the susceptible variety Little Club. Prior to the formation of teliospores each race was checked for purity at least once. The straws bearing the teliospores were cut into short segments and were thereafter frozen in the ice-cube compartments of a refrigerator, usually for periods ranging from a few months up to a year. Germination of the teliospores was induced by alternate wetting and drying, usually preceded by immersion in cold, flowing tap water for four or five days.

The uredial isolates in the progenies of selfed races were most frequently established from a random selection of individual aecia, but were sometimes derived from individual pustules of aecia or from randomly selected uredia arising from mass inoculations with aeciospores. Whatever the method employed, the idea of random sampling was kept in mind.

Culture work with rusts is so far removed from the pure-culture techniques employed with fungi that can be grown on artificial media in test tubes that it is inevitable that there should be some errors in the present work. The most likely sources of error are the following: contamination of a culture by another race prior to the formation of telia; transmission by insects of pycnial exudate from one barberry plant to another; contamination of one progeny culture by another when many are being studied at the same time. Despite such precautions as the segregation of uredial cultures in glass-walled compartments, and the protection of infected barberry plants by fly-proof screen

cages, it is unlikely that all these sources of error were circumvented. It seems probable that some of the races that occurred unexpectedly in certain progeny studies owe their origin to some of these sources of error.

Description of Inspection Types

In studies of the inheritance of rust characteristics, the infection types are regarded as visible characters of the rust organism. They are relatively constant under given environmental conditions but may be affected in their expression by changes in the environment of the host plant, particularly by changes in temperature. This environmental effect on the host–parasite complex is not regarded as an important source of error in the studies under discussion, as race identification was repeated if suspicion arose that any unusual environmental conditions were influencing the expression of the infection types.

The infection types are shown in Fig. 1. They may be described as follows:

0 No visible sign of infection.

0; Minute necrotic flecks but no uredia present.

1 Minute, round uredia, each surrounded by a necrotic area.

2 Somewhat larger, round uredia each in a chlorotic area usually surrounded by a necrotic band.

3 Medium-sized, round uredia surrounded by chlorosis.

4 Large, confluent uredia with little or no chlorosis.

x An indeterminate type of infection in which large and small uredia occur surrounded by various degrees of necrosis and chlorosis.

The signs ++, +, −, = are used to indicate deviation in the size of uredia from the normal for the type of infection, e.g. 3+ is a uredium somewhat larger than the usual type 3.

Interpretation of Data

Of the 42 cultures selfed only nine appeared to be homozygous for all pathogenic characters observed, namely races 1, 9, 36 (two cultures), 54, 69 (two cultures), 152, and 171 (Table I).

In some cultures there was observed a variation of a type that did not transgress the circumscribed limits of the physiologic race that was selfed, that is to say, the variants, though distinct from each other, must be regarded as merely substrains of the parent race. One example of this type of variation is to be found in the selfing of race 15A, Bagot Co., Que. The progeny showed at least four pathogenically distinct types all, however, within the limits of race 15. Another example is provided by race 177, the progeny of which showed two distinct types of infection on Reliance, both within the range of variability of that race. This "intra-race" type of variation occurred also in some of the cultures regarded as homozygous such as race 69, Carberry, Man., in the progeny of which there were noted slight differences in the size of uredia produced on Vernal.

FIG. 1. Infection types produced by physiologic races of *Puccinia graminis* var. *tritici* on differential hosts used for race identification. For description see text.

Of the 12 differential hosts used in the identification of the races only two, Little Club and Khapli, failed to show any appreciable variation in the infection types produced on them. Little Club, susceptible to all the parent races used in these studies, was uniformly susceptible to all progeny lines and is, for that reason, omitted from the tabulations of infection types. Khapli showed only minor variations in the infection types occurring on it, which ranged from small necrotic flecks to type 1 infection. It seems apparent that genes governing virulence to Khapli were not present in any of the races that were selfed and are probably rare in North American races of the rust.

On all the other differential hosts, more or less variation in infection type was noted in the progenies of certain races. In the following discussion the infection types occurring in the progenies are compared, for each variety, with the infection type produced by the parent race with the purpose of reaching some general conclusion as to the inheritance of the pathogenic properties of the rust.

TABLE I

RACES HOMOZYGOUS FOR PATHOGENICITY ON DIFFERENTIAL HOSTS, I.E., THE PROGENY WAS VIRTUALLY IDENTICAL WITH THE "SELFED" RACE

Race††	Infection types on differential hosts											Number of cultures in progeny
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
1	3+	0	3	0;	0;	0;	4	3	3	0;	1−	14*
9	4	0	4	4	4	4	4	4	3+	4	1	1**
36 No. 1	4	4	4−	0;	0;	0;	x	3+	3+	0;	1−	23†
36 No. 2	3±	3+	3	0;	0;	0;	x	3±	3	0;	1−	41†
54	3±	2	1	0;	0;	0;	1	3	3	1−	1	10**
69 No. 1	1	0	0;	0;	0;	0;	3±	3	3−	3+	1	11*
69 No. 2	1+	0	0;	0;	0;	0;	4	3+	3−	4−	1−	8**
152	2−	3−	0;	0;	0;	0;	x	3+	3+	0;	1−	25†
171	x	3=	1	0;	0;	0;	x−	4−	3	0;	1	2**

* *Progeny from separate pustules of aecia.*
** *Progeny from mass inoculation of aeciospores.*
† *Progeny from randomly selected single aecia.*
†† *Origin of races:*
Race 1. From a selfing of race 17 which, in turn originated from a selfing of race 21, Indian Head, Sask., 1934.
Race 9. A culture from barberry inoculated with sporidia from teliospores collected May, 1951, on Hordeum jubatum near Winnipeg, Man.
Race 36 No. 1. Duff, Sask., 1934.
Race 36 No. 2. Derived from a culture of race 17 which originated from the cross race 9 × race 36.
Race 54. Winnipeg, Man., 1949.
Race 69 No. 1. Carberry, Man., 1950.
Race 69 No. 2. Merrickville, Ont., 1950.
Race 152. Derived from a selfing of race 48, Ottawa, Ont.
Race 171. Origin uncertain.

TABLE II

RACES HETEROZYGOUS FOR PATHOGENICITY TO ONE OR MORE DIFFERENTIAL HOSTS. RACES VIRULENT TO THE DURUM DIFFERENTIAL HOSTS

Race**	Infection types on differential hosts											No. cultures
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
Par. 11	3+	4	4−	4−	4	4	4	3+	3+	0;	1	—
Prog. 11	3+	4−	4−	4−	4−	4−	4	3+	3+	0;	1	4
18	4−	4−	4−	1−	1−	1−	4	3+	3+	0;	1	2
Par. 15	4	4	4−	4−	4−	4	4	4−	4−	4−	1	—
Prog. 15	4	4	4	4	4−	4	4	4−	4−	4−	1	14
106	x	3	3	3±	x	x	x	3	3	3	1	2
52	3	3	3	0;	0;	0;	4	4−	3	3+	1	3
Par. 15A	3±c	3=	3=	4−	4−	4−	4	3	3	4−	1	—
Prog. 15A	3±c	4−	3−	4	4	4	4	3	3	4	1	2
15A	3±c	3=	3=	4	4	4	4	3	3	4	1	9
15A	3±	4c	4−	4	4	4	4	3−	3−	4	1	1
15A	4−c	3	3=	4	4	4	4	3	3	4	1	3
C. 52–2*	3±c	3	1−	3+	3+	3+	4−	3−	3−	3c	1	4
52	3±c	3=	3=	1	1	1	4	3±	3	4−	1	1
Par. 16	2−	2	2	4	4	4	4	4	1	1	1	—
Prog. 16	2	2	2	4	4	4	4	4	1	1	1	2
14	2+	2+	2+	4	4	4	4	4	4−	0;	1	1
23	2	2	2	1	1	1	4	4	4−	0;	1	1
Par. 17 No. 1	3+	0	3+	4−	4	4	4−	4−	3	0;	1	—
Prog. 17	3+	0	3+	4−	4−	4−	4−	4−	3	0;	1	20
1	3+	0	3+	0;	0;	0;	4−	4−	3	0;	1	9
38	2	3+	3−	x+	x−	x−	x−	4−	3	0;	1	1
148	3+	4−	3+	0;	0;	0;	1	3+	1	0;	1	1
Par. 17 No. 2	4−	0	3+	4−	4	4−	4−	4−	3+	0;	1	—
Prog. 17	4−	0	3+	4−	4−	4−	4−	4−	3+	0;	1	7
1	3±	0	3−	0;	0;	0;	4−	3+	3	0;	1−	1
136	x	0	3±	0;	0;	0;	4−	3+	3	0;	1	1
Par. 17 No. 3	4−	0	3+	4	4	4−	4−	4−	3+	0;	1	—
Prog. 17	3+	0	3−	4−	4−	4−	4−	4−	3+	0;	1	6
11	3+	4−	3+	4−	4−	4−	4−	4−	3+	0;	1	1
Par. 17 No. 4	3+	0	3−	4−	4	4−	4−	4−	3+	0;	1	—
Prog. 17	3+	0	3−	4−	4−	4−	4−	4−	3	0;	1	8
11	3+	3+	3−	4−	4−	4−	4−	4−	3	0;	1	4

TABLE II—(*Cont'd.*)

Race**	Infection types on differential hosts											No. cultures
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
Par. 21	4	0	4–	4	4	4	4–	4–	1	0;	1	—
Prog. 21	4	0	4–	4	4	4	4–	4–	1	0;	1–	2
17	4	0	3±	4	4	4	4–	4–	3+	0;	1–	27
11	4–	4–	4–	4–	4	4	4	4–	3+	0;	1	7
78	x+	0	3–	4–	4–	4–	4–	3+	3+	0;	1–	5
1	4	0	3–	1–	1–	1–	4–	3+	3+	0;	1–	10
136	x+	0	3–	1–	1–	1–	3±	3±	3+	0;	1–	2
176	4	0	4–	1–	1–	1–	4	4–	1	0;	1–	3
Par. 29	4–	0	3+	3±	x	x	x	3+	3	1–	1	—
Prog. 17	4–	0	3–	3±	4–	4–	4–	3±	3	1–	1	2
116	4–	0	4	4	4–	4–	3+	3+	1–	4	1	1
52	4–	3+	3+	0;	0;	0;	4–	3+	3–	4–	1–	2
18	3	3+	3	1	1	1	4	3+	3	0;	1	1
61	4–	0	3	1	1	1	x	3–	3–	1	1	1
57	4c	0	3	0;	0;	0;	4–	3+	3–	4–	1	2
Par. 38	2	3+	3–	4–	x	x	3±	4–	3	1–	1	—
Prog. 38	2	3±	3–	4–	x	x	3±	4–	3	1–	1	3
177	2	3±	3–	1–	1–	1–	3+	4–	3	1–	1	2
Par. 39	2	4	3–	4–	4	4	4	4–	3+	0;	1	—
Prog. 39	2	4	3–	4–	4	4	4–	4–	3+	0;	1	17
38	2	4–	3–	3±	x+	x+	4–	4–	3+	0;	1	1
177	2	4–	3–	1–	1–	1–	4–	3+	3	0;	1	1
Par. 48	1	0;	0;	4	x	x	x	4	3+	0;	1	—
Prog. 48	1	0;	0;	4	x	x	x+	4	3±	0;	1–	13
14	1	0;	0;	4	4	4	4	4	3±	0;	1–	12
151	1	3–	0;	4–	x	x	x	4–	3+	0;	1–	11
152	1	3–	0;	1	0;	0;	x	3+	3+	0;	1–	5
Par. 63	x	3–	3	4–	4	4	4	4–	1	0;	1	—
Prog. 63	x	3–	3–	4–	4	4	4–	4–	1	0;	1	18
C-44-1*	x	3–	3–	0;	0;	0;	4–	3+	1	0;	1	8
179	x	3	3	4–	4–	4–	4–	4–	3+	0;	1	2
Par. 73	x	0	3–	x	x	x	x	3±	3	1	1	—
Prog. 73	x	0	3–	x	x	x	x	3±	3	1	1	20
78	x	0	3–	4–	4–	4–	4–	4	3+	1–	1	5
113	x	3+	3–	x	x	x	x	3+	3+	0;	1	6
155	x	0	3–	1–	0;	0;	x	3+	3+	0;	1	8
193	x	4–	3–	0;	0;	0;	x	4	3+	0;	1	1

TABLE II—(*Concl'd.*)

Race**	Infection types on differential hosts											No. cultures
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
Par. 81	x	0	0;	4	4	4	4	4−	1	1−	1−	—
Prog. 81	x−	0	0;	4	4	4	4	4−	1	1−	1	7
88	x	0	0;	4	4	4	4	4	4−	1−	1	4
165	x	3=	0;	x	x	x	x+	x	3	4−	1−	1
169	x	0	0;	4	4	4	4	4	1	4	1	4
Par. 83	2	3−	0;	4	4	4	4	3+	3	3+	1	—
Prog. 83	2	3−	0;	4	4	4	4	3+	3−	3+	1	27
119	x−	0	0;	4	4	4	4	3+	3+	3+	1	1
Par. 87	3	3	3	3	x−	x−	x−	x	3	3	1	—
Prog. 87	3	3	3	3	x−	x−	x−	x	3	3	1−	2
C. 47–8*	3	3	3	3	0;	0;	x−	x	3	3	1−	18
C. 47–7*	4	4	4	4	x−	x−	x−	4−	1	x−	1	1
Par. 151	1	3−	0;	4−	x	x	x	4−	3+	0;	1−	—
Prog. 151	1	3=	0;	4−	x	x	x	3+	3+	0;	1−	11
113 ?	1+	3=	3=	4−	x	x	x	3+	3+	0;	1−	1

* *Races that do not fit any of the described races have been given accession numbers as indicated.*

** *Origin of races:*

Race 11. Derived from a selfing of race 21, Indian Head, Sask., 1934.

Race 15. Normandin, Que., 1946.

Race 15A. Bagot Co., Que., 1947.

Race 16. Origin doubtful. Appeared as admixture, possibly through mutation, in a culture of race 98.

Race 17 No. 1. Elm Creek, Man., 1936.

Race 17 No. 2. From uredia on barberry infected with race 21, Indian Head, Sask., 1934.

Race 17 No. 3. From uredia on a barberry infected with a culture of race 17 which, in turn, arose from a selfing of race 21, Indian Head, Sask., 1934.

Race 17 No. 4. From a selfing of a monoaecial culture of race 21 which was obtained from a selfing of race 21, Indian Head, Sask., 1934.

Race 21. Indian Head, Sask., 1934.

Race 29. Guelph, Ont., 1950.

Race 38. Oak Lake, Man., 1931.

Race 39. Dufrost, Man., 1931.

Race 48. Ottawa, Ont.

Race 63. Claresholm, Alta., 1942.

Race 73. Lethbridge, Alta., 1938.

Race 81. Charlottetown, P.E.I., 1938.

Race 83. From barberry, Winnipeg, Man., 1928.

Race 87. Morden, Man., 1945.

Race 151. Derived from a selfing of race 48, Ottawa, Ont.

TABLE III

RACES HETEROZYGOUS FOR PATHOGENICITY TO ONE OR MORE DIFFERENTIAL HOSTS. RACES NONVIRULENT TO THE DURUM DIFFERENTIAL HOSTS

Race‡‡	Infection types on differential hosts											No. cultures
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
Par. 2 (white)	2−	2	2	0;	0;	0;	x	3	3	1	1	—
Prog. 2 (white)	2	2	2	0;	0;	0;	x	3	3	1	1	1
65 (white)	2	2	2+	0;	0;	0;	4−	3+	3	4−	1	4
Par. 49	3	0	3	0;	0;	0;	x	3±	1	0;	1−	—
Prog. 49	3−	0	3	0;	0;	0;	x	3±	1	0;	0;	7
80	3	0	3	0;	0;	0;	x	3±	1	3±	1−	11
36	3	3+	3	0;	0;	0;	x	4−	3+	0;	1	1
97	3	3	3	0;	0;	0;	x−	3±	1	3+	0;	1
52	3	3	3	1−	0;	0;	x	3±	3	3	1	1
61	3	0	3	0;	0;	0;	x	3±	3	0;	1	8
125	3	3	3	0;	0;	0;	x	3±	1	0;	1	1
82	3+	3+	3−	0;	0;	0;	x	4−	3	x	1	1
Par. 56 No. 1	4	4−	3+	0;	0;	0;	4−	3±	0;	0;	1	—
Prog 56	4−	4−	3	0;	0;	0;	4−	3±	0;	0;	0;	16
52	4−	3+	3±	0;	0;	0;	4−	3±	3	4−	1−	6
147	4−	3+	3	0;	0;	0;	4−	3	0;	4−	1−	3
Par. 56 No. 2	4	4	4−	1−	1−	1−	4	3±	1	0;	1−	—
Prog. 56	4	4	4	1−	1−	1−	4	3	1−	0;	1−	12
147	4	4	4	0;	0;	0;	4	3	0;	3	0;	5
18	4	4	4−	0;	0;	0;	4	3+	3	0;	1−	2
52	4	4	4	0;	1	1	4	3+	3	4	1−	2
117	4c	2−	0;	4	4	4	4	3+	3	4−	1	1
34	4c	4	3	4	4	4	4	4−	1	1−	1	1
C. 52-1*	x+	4	x	4	4	4	4	3+	3	4	1	1
Par. 125	4	4	3+	1−	0,	0;	x	4	1	0;	1	—
Prog.125	4	4	3+	1−	0;	0;	x	4−	1−	0;	1−	4
56	4	4	3+	1−	0;	0;	4−	4−	1−	0;	1−	4
36	4	4	3+	1−	0;	0;	x	3+	3+	0;	1−	5
146	4	4	3+	1−	0;	0;	x	3+	1−	x−	1−	7
147	4	4	3+	1−	0;	0;	4−	4−	1−	3+	1−	4
148	4	4	3+	1−	0;	0;	0;	3+	1−	0;	1−	1
Par. 59	2	0	1−	0;	1−	1−	4	3+	4−	0;	1−	—
Prog. 59	2	0	1	1−	1	1	4	3+	3+	1	1	12
69	2	0	2+	1	1	1	4	3+	3	4−	1	5
27	2	0	0;	0;	0;	0;	x	3+	1	4−	1	1
Par. 59A	2	2	0;	0;	0;	0;	4−	3	3	0;	1	—
Prog. 59A	2	2	0;	0;	0;	0;	4−	3	3	0;	1−	4
69	2	0	0;	0;	0;	0;	4−	3	3	4	1−	3

TABLE III—(*Concld.*)

Race‡‡	Infection types on differential hosts											No. cultures
	Ma.	Rel.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.	
Par. 80 No. 1	4	0	4	1−	1−	1−	4	4−	1	4−	1	—
Prog. 80	3+	0	3	1−	1−	1−	4−	4−	1	3+	1−	19
52	3+	4−	3±	1−	1−	1−	4−	4−	3+	3+	1−	1
Par. 80 No. 2	3+	0	3−	0;	0;	0;	3+	3	1−	3	1	—
Prog. 80	3+	0	3−	0;	0;	0;	3	3	1−	3	1	3
147	4	4−	4	1	1	1	4	4−	1	4	1	2
Par. 139	2+	0	2+	1−	1−	1−	x	4	1−	1−	1	—
Prog.139	2+	0	2	0;	0;	0;	x	3+	0;	1	1−	1
140	2+	0	3=	1−	0;	0;	x	4−	3	0;	1−	2
23	2	0	1+	0;	0;	0;	4	3+	3	0;	1	1
27	2+	0	2	0;	0;	0;	x	4−	0;	4−	1−	3
C. 52–3*	2	3	1	0;	0;	0;	x	3−	0;	0;	1−	1
C. 52–4*	2	3	1	0;	0;	0;	0;	3−	0;	x	1=	1
Par. 157	x	0	3=	1−	0;	0;	4−	4−	3	x−	1	—
Prog. C. 49–1*	x	3=	3=	1−	0;	0;	x=	4−	3	0;	1	4
171 ?**	x	3=	0;	1−	0;	0;	1	4−	3	0;	1	2
35 ?†	4−	3=	0;	1−	0;	0;	1	4−	3	0;	1	1
118 ?††	x	0;	0;	1−	0;	0;	1	4−	3	0;	1	3
61	4−	0;	3=	1−	0;	0;	x−	4−	3	0;	1	2
54 ?‡	3±	0	1+	1−	0;	0;	x	4−	3	0;	1	2
173	2	3	3=	1−	1−	1−	4−	3	1	1	1	1
Par. 173	2	4−	3−	0;	0;	0;	4	4−	1−	0;	1	—
Prog.173	2+	4−	3=	0;	0;	0;	x	4−	1	0;	1	13
177	2+	4−	3=	0;	0;	0;	x	4−	3−	0;	1	2
56	4	4−	4−	1	1−	1−	4−	4−	1	0;	1	1
Par. 177	1	3=	3=	0;	0;	0;	4	4	4−	0;	1	—
Prog.177	1	3=	3=	0;	0;	0;	4	4−	4−	0;	1	9
177	1	4	3=	0;	0;	0;	4	4−	4−	0;	1	4
Par. 192	4−	4−	4−	4	0;	0;	0;	3±	1	x−	1	—
Prog.192	4−	4−	4−	4	0;	0;	0;	3±	1	x−	1	21
C. 47–1*	4	4	4−	4−	0;	0;	0;	3	1	4	1	19
C. 47–2*	4	4	4−	0;	0;	0;	1−	3	1	x−	1	3
C. 47–3*	4	4	4−	4−	0;	0;	1−	3	3	x−	1	4
C. 47–4*	4−	4	4−	0;	0;	0;	0;	3−	1	4	1	5
C. 47–5*	3+	4−	3+	0;	0;	0;	0;	3−	3	x−	1	1

NOTE: *Footnotes to Table III are given on p. 515.*

Infection Types on Marquis

There was a strong tendency for the progeny cultures to resemble the parent races in their infection types on Marquis. The absence of clear-cut segregation of rust characteristics on this host is in sharp contrast to the behavior of the rust on most of the other varieties. Such variation as was observed was usually rather indefinite or, at least, decidedly limited in range. This behavior of the rust (on this host) in selfing studies is in conformity with that previously recorded in crosses between races (3, 4), i.e. a tendency for the hybrid rust to resemble the maternal parent race. In the absence of any other explanation, this phenomenon has been attributed to the influence of the cytoplasm of the maternal parent race. As would be expected, this phenomenon, which, in crosses, tends to make reciprocal hybrids diverge in pathogenicity, has the contrary effect in the selfing of races where it tends to limit divergence or variation. Peculiarly, this supposed cytoplasmic effect is observable only in the behavior of the rust on Marquis and, to a lesser extent, on Kota.

Infection Types on Reliance

Earlier work (2) had indicated that type 0 infection (inability to produce rust) was dominant to type 4 infection (large uredia). Of the 19 cultures, in the present study, that produced type 0 infection on Reliance six were apparently homozygous for this character, producing type 0 in all the progeny. Nine cultures were heterozygous, producing races with 0 and 4 or 3+ types of infection. Four cultures showed segregation of the progeny into classes of type 0 and type 3 or 3−. Type 0 is apparently dominant to type 3 as well as type 4.

Four of the parent cultures produced type 2 infection on Reliance. Three of these were homozygous for that character. In one culture (race 59A) types 2 and 0 were both present in the progeny.

Footnotes to Table III

* *Races that do not fit any of the described races have been given accession numbers as indicated.*
** *Should be* x− *on Kub. to fit race 171.*
† *Should be* 3+ *on Kub. to fit race 35.*
†† *Should be* 4 *on Kub. to fit race 118.*
‡ *Should be* 1 *on Kub. to fit race 54.*
‡‡ *Origin of races:*

Race 2. A culture with white uredia, derived from a "selfing" of race 155 which originated in the "cross" race 36 white × race 120.
Race 49. Star City, Sask., 1941.
Race 56 No. 1. Arnes, Man., 1934.
Race 56 No. 2. Mindemoya, Ont., 1950.
Race 125. Durban, Man., 1934.
Race 59. Creston, B.C., 1950. The aeciospores from a number of the aecial pustules produced, on wheat seedlings, infections containing abortive pycnia instead of uredia. This phenomenon has been described elsewhere (1).
Race 59A. Virginia, U.S.A.
Race 80 No. 1. Grand Point, Man., 1941.
Race 80 No. 2. Ottawa, Ont., 1949.
Race 139. Yorkton, Sask., 1934.
Race 157. Derived from the "cross" race 36 × race 120.
Race 173. Derived from the "cross" race 39 × race 125.
Race 177. Creston, B.C., 1943.
Race 192. Ottawa, Ont., 1941.

Of the eight cultures with type 3 infection, four were homozygous for this character, three were heterozygous, producing both 3 and 4 types in the progeny, while one culture (race 83) produced in its progeny 27 isolates with type 3 infection and one isolate with type 0.

Of the 11 cultures with type 3+ or 4 infection, 10 produced progeny that, in this respect, closely resembled the parents. In the progeny of one of the selfed cultures (race 56, Mindemoya) a type 2 infection appeared in one of 24 isolates.

In general, these results accord with experience in crossing races. Types 3 and 4 appear to be recessive to type 0.

Infection Types on Kota

The inheritance of infection types, as expressed on Kota, follows the pattern already shown on Marquis—a marked resemblance of the progeny to the parent race. The seven parent cultures with type 0 produced progenies with the same type of infection with the exception of one isolate out of 12 in race 151. In the selfing of cultures with type 1 or type 2 infection, the progenies showed only a narrow range of variation, most pronounced in race 59 (range 0; to 2+) and race 139 (range 1 to 3=). Cultures with infection type 3− produced mostly the same type of infection in the progenies, though type 4 infection also occurred in some progenies as of races 15A, 80, 87, and 173. Races 15A and 157 were exceptional in that their progenies contained also some isolates with type 1 and (in race 157) type 0 infection. The progenies of races with types 3+ and 4 infection showed only slight deviations from the infection types of the parent races except for race 56 (Mindemoya) in which one out of 24 isolates showed infection type 0.

Infection Types on Arnautka, Mindum, and Spelmar

Earlier studies (2) had indicated that type 4 infection was dominant to types 1 and 0. The studies reported here show that this conclusion is applicable to the races dealt with in the present paper and is probably applicable to stem rust races in general.

Eight of the cultures producing type 4 infection on Arnautka, Mindum, and Spelmar were heterozygous for this character. Admittedly, the populations studied were rather small for submission to statistical analysis. It may be mentioned, however, that of seven randomly selected progenies five showed a good fit to a 3 : 1 ratio. This result confirms previous conclusions (2) that pathogenicity on these varieties is conditioned by a single pair of genes.

Races with types 0 or 1 infection on all three varieties produced progenies that resembled the parent races in this respect. This is applicable to races 1, 2, 36, 49, 54, 56, 125, 59, 59A, 69, 80, 139, 152, 157, 171, 173, and 177. Race 56 (Mindemoya) is an exception; in the progeny of this race, there were three out of 24 isolates with type 4 infection on Arnautka, Mindum, and Spelmar. It is thought most likely that this deviation from the general rule was due to undetected impurities in the parent culture.

The only race studied which produced type 4 infection on Arnautka but type 0 on Mindum and Spelmar was race 192. In the progeny of this race, 44 isolates resembled the parent (Arn. 4, Mnd. 0, Spm. 0) whereas nine isolates produced type 0 on all three varieties. Evidently there was present in race 192 a dominant gene that governs virulence on Arnautka but is ineffective on Mindum and Spelmar.

Races with x type infection on Mindum and Spelmar were heterozygous, producing in their progenies types 4 and 0 as in the selfing of race 29, or x and 0 as in the selfing of races 38 and 87, or 4, x, and 0 as in the case of races 48 and 73. The x type is perhaps a reflection of incomplete dominance.

Complete dominance was shown in races such as 11, 15, 15A, 16, 17, 39, 63, and 81 which, in their progenies, produced 4 and 1 types or 4, x, and 1 types. It may be noted that the several selfings of race 17 show that this race contains strains phenotypically alike but genotypically different.

Infection Types on Einkorn

The results of the selfing studies make it evident that type 1 infection is dominant to type 3. All the selfed races with type 1 infection, except possibly race 80, appeared to be heterozygous for this character, producing in their progenies both 1 and 3 types of infection. Of the 29 parent cultures with type 3 infection, 24 were homozygous, all the progeny resembling the parent race in this respect. The remaining five races (races 17, 29, 59, 87, 157) produced mostly type 3 infection in the progeny but each produced also a single isolate with type 1 infection. Despite these exceptions, it may be stated as a general rule that races with type 3 infection are homozygous for that character.

Infection Types on Vernal

In crosses between races it has been shown that the 4 type of infection on Vernal is recessive to the 0 and 1 types (2). The results of the selfing studies reported here are in conformity with this finding according to which races with type 3 or 4 infection on Vernal should be homozygous for that character. The nine cultures virulent on Vernal produced progenies of the same kind except for the presence, in the progeny of race 87, of a single isolate with type x infection.

Of the cultures that produced 0 or 1 type, 21 were homozygous and 10 were heterozygous—three producing 0, x, and 4 infection types in the progeny and seven producing 0 and 4 types only. The progenies of five of these seven cultures were randomly selected and were subjected to statistical analysis. Four of these conformed to a 9 : 7 ratio (type 0: type 4) indicating Mendelian inheritance with two pairs of genes involved. It may be mentioned in this connection that earlier studies (2) had indicated that in the cross race 9 × race 36 there were two pairs of genes involved. The ratio in that cross, however, was 15 : 1.

Only two of the selfed races were characterized by type x infection on Vernal. Race 157, which gave an x− type, did not show any sign of this infection type in the progeny, all of which was recorded as type 0 or 1. Race 192, a race with a more characteristic x type infection, produced a progeny consisting of 29 isolates with type x and 24 with type 4 infection—a good fit to a 9 : 7 ratio.

Genetical Constitution of Races

Since the work here reported was not primarily designed as a genetical study, many of the F_2 populations were too small to permit the calculation of genetical ratios. The results in a number of the studies are, however, conclusive enough to justify some speculation on the factorial bases of the inheritance of infection types, particularly on the varieties Reliance, Arnautka, Mindum, Spelmar, Einkorn, and Vernal. Fig. 2 shows the phenotypes and the conjectured genotypes of race 49 and of the races identified in the progeny resulting from its selfing. The scheme of inheritance postulated for this race and its derivatives may be applied in many of the other selfing studies though it should be noted that in the absence of calculated ratios it is not possible to state that the inheritance of the various characters follows exactly the scheme indicated in Fig. 2.

The Testing of Resistant Wheat Varieties with Mass Progeny Cultures

One of the possibly important sources of virulent rust characteristics is the presence in a rust race of recessive genes governing virulence. A race possessing such recessive genes may not, itself, be virulent to a given wheat variety but it may produce, when selfed, races that are virulent to it. The existence in rust races of such hidden pathogenic potentialities may be investigated by testing resistant wheat varieties simultaneously with (i) a known physiologic race and (ii) the progeny of the selfing of that race. In the work here reported, it was assumed that virulent traits could be detected by inoculating resistant wheats with aeciospores derived from barberry plants infected with given races or with urediospore cultures arising from the aeciospores.

The following 12 physiologic races were investigated in that manner: 15A, 21, 29, 52, 56, 63, 90, 98, 102, 155, 171, and 192. The data derived from these tests are summarized in Table IV.

In addition to the differential hosts, the following wheats were inoculated: *common wheat*—McMurachy, Kenya R.L. 1373, Kenya × Gular, Gabo, Red Egyptian, Lee, Frontana × (R.L. 2265 × Redman[2]) R.L. 2520, Timstein × Newthatch C.I. 1264; *durum wheat*—Iumillo; *Triticum timopheevi.*

The reaction of the differential hosts showed that eight of the 12 races used were heterozygous for pathogenicity, namely, races 15A, 21, 29, 56, 63, 90, 98, and 192.

The wheats listed above showed a rather high seedling resistance in all the tests irrespective of whether the rust was derived from a homozygous or a

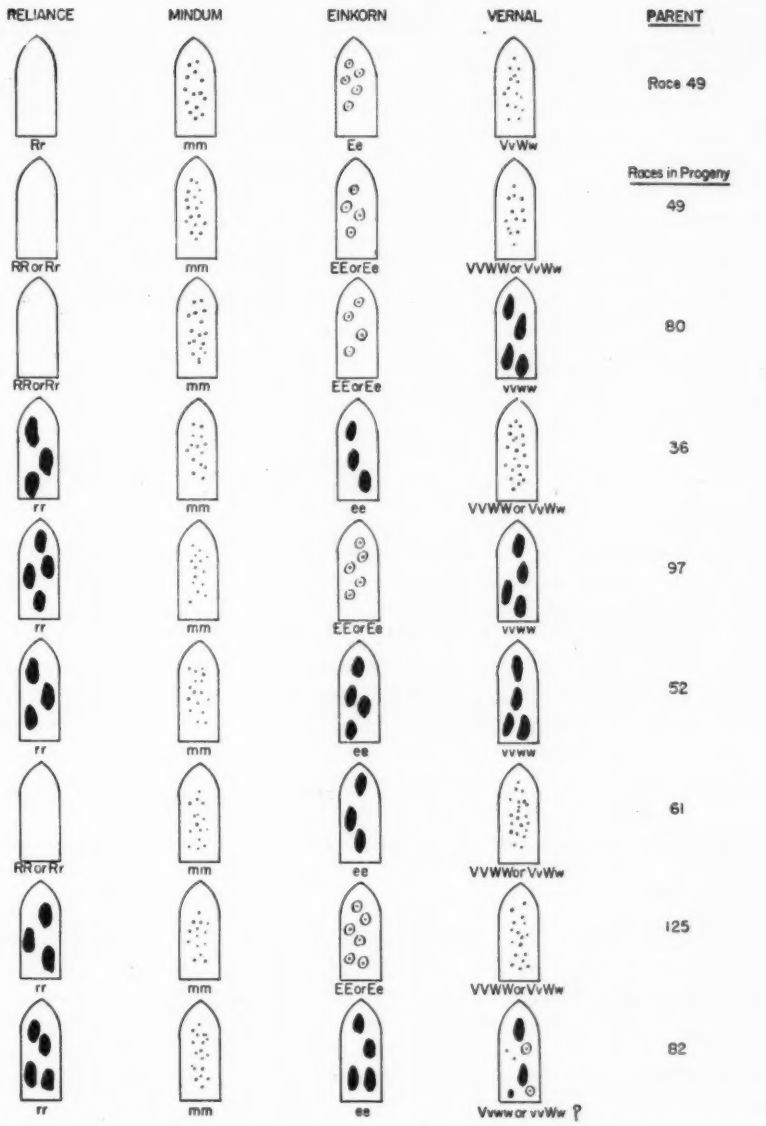


FIG. 2. Hypothetical genetical constitutions of race 49, Star City, Sask., 1941, and of its progeny with respect to infection types on certain differential hosts.

It is assumed that a dominant gene *R* governs inability to produce rust on Reliance; that a dominant gene *M* governs ability to produce large pustules (4 type) on Mindum, Arnautka, and Spelmar; that a dominant gene *E* governs ability to produce small pustules (1 type) on Einkorn; and that two dominant genes *VW* govern ability to produce small necrotic flecks on Vernal.

TABLE IV

INFECTION TYPES ON RESISTANT WHEAT VARIETIES INOCULATED WITH MASS CULTURES OF UREDIOSPORES DERIVED FROM AECIOSPORES OF 12 PHYSIOLOGIC RACES

Variety	Physiologic races											
	15A*	21**	29**	52**	56**	63**	90†	98**	102**	155**	171**	192†
McMurachy	0;	0;	0;	0;	0;	0;	0;	0;	1–	1–	0;	1–
Kenya R.L. 1373	0	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1–
Kenya × Gular	X–	0;	1–	1–	1–	0;	1–	1+	0;	0;	0;	X–
Gabo	1	0;	0;	1–	1–	1–	1–	1	0;	0;	1–	1–
Iumillo	0;	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;
Triticum timopheevi	0;	0;	0;	0;	0;	0;	0;	0;	0	0	0;	0;
Lee	1	1–	1–	1–	1–	1–	1–	1–	0	0;	1–	1–
R.L. 2520	0;	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;
Red Egyptian	0;	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;
Timstein × Newthatch	1–	1–	1–	1–	1–	1–	1–	1	0	0;	1–	1

* *On the varieties below, the parent race produced infection types almost identical with those produced by the progeny except on Kenya × Gular on which the parent race produced type 1 infection.*

** *On the varieties below, the parent race produced infection types almost identical with those produced by the progeny.*

† *Parent race not tested on the varieties below.*

TABLE V

INFECTION TYPES ON RESISTANT WHEAT VARIETIES INOCULATED WITH MASS CULTURES OF UREDIOSPORES DERIVED FROM AECIOSPORES OF SIX PHYSIOLOGIC RACES

Variety	Physiologic races					
	2*	16	83	139	157	171
McMurachy	0;	0;	0;	0;	0;	0
Kenya R.L. 1373	0;	0;	0;	0;	0;	0
K. 117A	2–	2–	1–	2–	2–	1–
K. 58 F. (L)	0	0;	0	0;	0;	0;
K. 338	1–	0;	1–	1	0;	0;
Lee	0;	0;	1	1–	0;	0;
Carleton	1 and 4	0;	4	0;	0;	0;

* *A strain with white uredia.*

heterozygous race. The only indication of greater virulence in the progeny than in the parent race occurred on the variety Kenya × Gular which displayed an x− type of infection to the progeny of race 15A but a 1 type to the parent race.

In another test, recorded in Table V, the same procedure was followed except that the varieties were not inoculated simultaneously with the parent rust cultures. Earlier tests of these varieties with the parent cultures were available for comparison. As in the preceding test, there were no appreciable differences between the reactions of the varieties to the parent races and their progenies except for the durum wheat Carleton which showed a 1 type of infection when inoculated with race 2 (white) but 1 and 4 types when inoculated with the progeny culture. Isolations from the 4 type infections showed that the race responsible for them was race 65, a race with white uredia.

The apparent absence in these races of genes for high virulence to the rust-resistant common wheats in these tests does not necessarily indicate that such genes are absent from North American stem rust races, but it does suggest that such genes are not at all common.

Discussion

Apart from the 0 type of infection on the durums Arnautka, Mindum, and Spelmar, which seems to be inherited as a unit, and the 4 type of infection on these varieties which is similarly associated, there is little indication that infection types on any two of the differential hosts are governed by the same gene or by very closely linked genes. The apparently independent inheritance of the characters expressed on the various hosts is a factor favoring the production of a great variety of pathogenic strains in the progenies of races that happen to be heterozygous for several of these characters.

If a race happens to display dominant characters (infection types) on several differential hosts it is impossible to forecast with certainty the nature of the progeny because these characters may be present in either a homozygous or a heterozygous state. Race 21, for example, shows the dominant characters 0 type on Reliance, 4 type on durums, 1 type on Einkorn, 0 type on Vernal. It could conceivably be homozygous for all or it might be heterozygous for all. In the selfing here reported it appears to have been heterozygous for the infection types on Reliance, the durums, and Einkorn but homozygous for infection type on Vernal. The progeny of different cultures of race 21 may, obviously, differ greatly in racial composition depending on which of these characters happen to be in a homozygous condition.

It is only when a number of recessive characters occur in a race that any accurate prediction of the progeny is possible. It is reasonably certain that a race such as race 52, which possesses the recessive characters 4 type on Reliance, 0 type on the durums Arnautka, Mindum, and Spelmar, 4 type on Einkorn, and 4 type on Vernal, will merely reproduce itself in a selfing study.

The production of characters of increased virulence in the progeny of a race is, of course, dependent on the parent race containing a dominant avirulent

character. Race 56, having two such characters, 1 type on Einkorn and 0 type on Vernal, has a tendency to produce races of greater virulence on Einkorn and Vernal, such as races 147 virulent on Vernal and race 52 virulent on both Einkorn and Vernal. It is scarcely necessary to point out that such recessive virulent characters, which come to light in selfing studies, tend to be suppressed in crosses with races possessing the corresponding avirulent characters.

Acknowledgment

The author wishes to express his gratitude to Dr. R. F. Peterson, Officer-in-Charge, Cereal Breeding Laboratory, Winnipeg, Man., for his critical reading of the manuscript.

References

1. JOHNSON, T. and GREEN, G. J. The production by *Puccinia graminis* of abortive pycnia on wheat. Can. J. Agr. Sci. In press.
2. JOHNSTON, T. and NEWTON, M. Mendelian inheritance of certain pathogenic characters of *Puccinia graminis tritici*. Can. J. Research, C, 18 : 599-611. 1940.
3. JOHNSTON, T., NEWTON, M., and BROWN, A. M. Further studies of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 14 : 360-373. 1934.
4. NEWTON, M., JOHNSON, T., and BROWN, A. M. A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 10 : 775-798. 1930.

PHOSPHORYLASE IN THE CHLOROPLASTS OF WHEAT[1]

By Michael Shaw[2]

Abstract

Phosphorylase activity was demonstrated in the chloroplasts of wheat, bean, and sunflower by incubating living starch-free sections with glucose-1-phosphate in acetate buffer at pH 5.8–6.0.

Introduction

So far as is known, starch formation *in vivo* is restricted to the plastids. Thus the discovery of starch phosphorylase in plants (2) was followed by histochemical studies of its distribution employing the technique of incubation with glucose-1-phosphate (6, 7, 1, 5). Yin (6, 7) concluded that phosphorylase was localized in the plastids in leaves (stomatal and mesophyll cells) as well as in seeds and roots. On the other hand Stocking (5), having failed in both histochemical and biochemical tests to demonstrate phosphorylase activity in the chloroplasts of potato, chard, and sunflower, concluded that his results pointed to a lack of phosphorylase in the chloroplasts and to its presence outside them.

Paech and Krech (3), by avoiding the use of frozen or anesthetized sections (1, 5), were able to demonstrate the occurrence of phosphorylase in leucoplasts and in the chloroplasts of stomatal cells as well as the mesophyll chloroplasts of wheat. Unfortunately no data were presented to illustrate phosphorylase activity in mesophyll chloroplasts and it is difficult to agree with their statement that Stocking's Figs. 1-3 can be interpreted as indicating this.

Theoretical Considerations

A large number of observations showed that it was impossible to plasmolyze any cell in which subsequent iodine staining revealed the synthesis of polysaccharide from glucose-1-phosphate due to phosphorylase activity. The ester was toxic to living cells in the concentrations (0.2%) normally employed and evidently only penetrated in amounts sufficient to cause polysaccharide synthesis after the destruction of semipermeability. It also seemed possible that glucose-1-phosphate might destroy semipermeability at the cell surface without at first affecting that at the chloroplast surfaces, since some cells were rendered incapable of plasmolysis before they formed polysaccharide. Assuming strict localization of phosphorylase within the plastids, synthesis would then be dependent on the disorganization of the chloroplast membrane by the ester itself and might therefore be expected to occur independently in the

[1] *Manuscript received March 19, 1954.*
Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan.

[2] *Associate Professor of Biology (Plant Physiology).*

individual plastids of a single cell. It had in fact been observed repeatedly that polysaccharide formation in stomata often occurred in proximity with the chloroplasts at one end but not at the other end of a single guard cell. If the absence of interfering factors (e.g. β-amylase or inhibitors of phosphorylase, see (4)) be assumed, then it follows that whether or not the polysaccharide will be found within the chloroplasts will depend upon two circumstances, viz.: (1) the relative rates of diffusion of glucose-1-phosphate into and of the water soluble enzyme out of the chloroplasts and (2) the diffusibility of the synthetic polysaccharide, which is also water soluble. Both the enzyme and its product might diffuse to and be adsorbed at secondary cytoplasmic loci following disruption of semipermeability at the chloroplast surface.

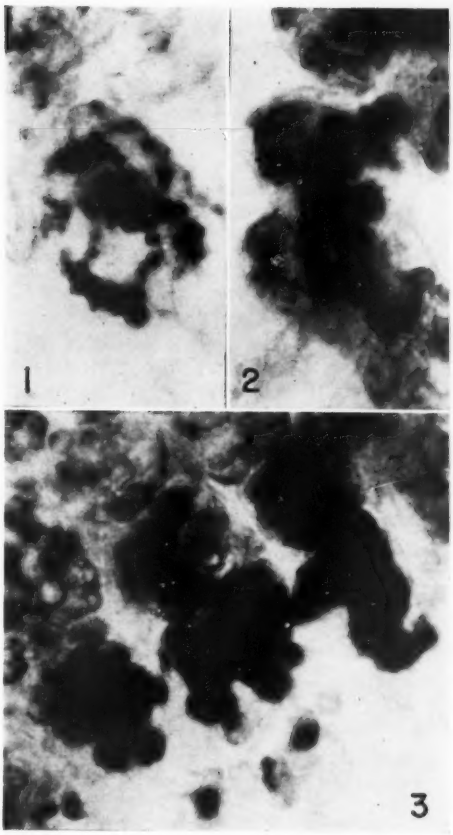
Method

The foregoing considerations suggested that only incubation of sections initially alive could bring the glucose-1-phosphate into contact with completely intact chloroplasts. Clearly, this could not be accomplished by the use of frozen sections or sections anesthetized to the point of irreversible destruction of semipermeability. In the absence of suitable fixation procedures incubation of living material therefore appeared to offer the best chance of demonstrating phosphorylase activity within the chloroplasts if this actually occurred. Accordingly, thin (*circa* 20μ) freehand sections of starch-free leaves were floated on 0.2% glucose-1-phosphate (potassium salt, obtained from Nutritional Biochemicals) in $M/40$ acetate buffer at pH 5.8–6.0 in shallow dishes. The dishes were subjected to a reduced pressure of 20–30 mm. Hg which was abruptly released two or three times to ensure contact between cell surfaces and the solution. They were then allowed to stand on the laboratory bench and sections were removed at approximately half hour intervals for four to six hours. Immediately on removal each section was examined microscopically in a drop of the incubation medium. Dilute iodine – potassium iodide was then drawn under the edge of the coverslip. The synthetic polysaccharide produced by phosphorylase activity stained a deep blue color. Addition of fluoride (0.01 M.) did not inhibit synthesis and control sections incubated with glucose gave negative iodine tests.

Results

Phosphorylase activity was usually evident after one to two hours of incubation. The most common result was the formation of irregular masses of polysaccharide in the cytoplasmic matrix around the chloroplasts (Fig. 1). In some cells, however, it was clear that synthesis was essentially confined either to the surface of or within the chloroplasts. A typical result, obtained with mature leaves of wheat (*Triticum aestivum* L. emend. Fiori et Paoletti. var. Onas), is shown in Figs. 2 and 3. Similar results were observed with starch-free sections of sunflower (*Helianthus annus* L.) and broad bean (*Vicia faba* L.). In the living mesophyll cell of wheat the chloroplasts are closely

PLATE I



FIGS. 1, 2, and 3. Photomicrographs of freehand sections of Onas wheat incubated four hours in 0.2% glucose-1-phosphate in acetate buffer at pH 5.8, and stained with aqueous iodine to show polysaccharide formed by phosphorylase activity. FIG. 1. Formation of irregular masses of polysaccharide in the cytoplasmic matrix. FIGS. 2 and 3. Polysaccharide synthesis in the chloroplasts. Note hexagonal pattern, streaking due to diffusion, and independent synthesis in adjacent chloroplasts.

adpressed so that each appears roughly hexagonal in outline and the hyaline chloroplast membranes are clearly visible. Even on cursory inspection a hexagonal pattern was evident in the formation of the synthetic polysaccharide as shown in Figs. 2 and 3. Moreover, synthesis was clearly independent in individual chloroplasts since stained and unstained plastids were often juxtaposed in a single cell (Fig. 3). Close inspection also revealed clear evidence of diffusion of the iodine-stained polysaccharide away from the centers of synthesis (Figs. 2 and 3). Finally, apart from the 'streaking' due to diffusion there was no evidence of phosphorylase activity in the cytoplasmic matrix in those cells in which the enzyme was clearly associated with the plastids as in Figs. 2 and 3.

Discussion

The results provide an unequivocal demonstration of the association of phosphorylase activity with the chloroplasts of mesophyll cells. Since starch formation *in vivo* occurs within the chloroplast membrane the enzyme probably also occurs within the chloroplast and the results reported here are consistent with this opinion. It is further suggested that the apparent activity of phosphorylase outside the chloroplasts is an artifact due to cellular disorganization and to diffusion, possibly of both the enzyme and its product away from the chloroplast centers. These conclusions support those of Yin (6, 7) and Paech and Krech (3) but stand in opposition to that of Stocking (5).

Dyar (1) reported that phosphorylase was associated with the nuclei of meristematic cells in roots. Paech and Krech (3) suggested that this association was an artifact due to the aggregation of plastids around the nucleus. They found no activity in the nuclei of guard cell initials. An apparent association of phosphorylase activity with the nuclei in guard cells of *Vicia* was observed by the writer, but only under conditions which also caused the chloroplasts to aggregate around the nucleus. It is therefore concluded that incubation with glucose-1-phosphate provides no critical evidence for the occurrence of phosphorylase outside the plastids in living cells.

Some further interest attaches to the demonstration of phosphorylase activity in wheat chloroplasts, since these do not normally form starch. The observations reported here were first made on wheat grown in the greenhouse in June and July. Attempts to repeat them on two occasions during the winter months failed completely since no phosphorylase activity whatsoever was detected anywhere except in the guard cells. This may have been due to one or more of a number of factors (4). Similarly, the writer found that starch accumulated around infections of *Puccinia graminis* on a number of varieties of wheat during the summer and early fall but not during the winter months. These observations on wheat suggest a seasonal variation in phosphorylase activity, or in factors which obscure it, which may be associated with illumination during the growing period.

References

1. DYAR, M. T. Some observations on starch synthesis in pea root tips. Am. J. Botany, 37 : 786-791. 1950.
2. HANES, C. S. The breakdown and synthesis of starch by an enzyme system from pea seeds. Proc. Roy. Soc. (London), B, 128 : 421-450. 1940.
3. PAECH, K. and KRECH, E. Über die Stärkebildung in den Plastiden. Planta, 41 : 391-395. 1953.
4. PORTER, H. K. The inhibition of plant phosphorylases by β-amylase and the detection of phosphorylase in barley. Biochem. J. 47 : 476-482. 1950.
5. STOCKING, C. R. The intracellular location of phosphorylase in leaves. Am. J. Botany, 39 : 283-287. 1952.
6. YIN, H. A. Phosphorylase in plastids. Nature, 162 : 928-929. 1948.
7. YIN, H. A. and SUN, C. N. Localization of phosphorylase and of starch formation in seeds. Plant Physiol. 24 : 103-110. 1949.

AN ANATOMICAL STUDY OF CANE AND CROWN GALLS[1]

By W. E. McKeen[2]

Abstract

An anatomical study was made of galls which occur naturally on the stems of boysenberry and Himalaya blackberry plants. The galls form at any region of the stem of the floral canes of plants systemically infected with *Agrobacterium rubi* and originate from or near the cambial layer, and not from the outer layer of the pericycle. The vascular system in galls caused by *A. rubi* and *A. tumefaciens* on broad bean, tomato, sugar beet, loganberry, and Himalaya blackberry plants is connected to the vascular system of the organ on which they are growing.

Introduction

While conducting a general investigation of the cane and crown gall diseases caused by *Agrobacterium rubi* (Hildebrand) Starr & Weiss, 1943, and *A. tumefaciens* (E. F. Smith & Towns.) Conn, 1942, a study was made of the anatomical structure of the galls and the tissues from which they arise. Galls occurring naturally on the boysenberry (*Rubus loganobaccus* hybrid) and the Himalaya blackberry (*R. procerus* P. J. Muell.) were available in all stages of development and proved very suitable for study. Certain features concerned in gall formation were revealed with unusual clarity in these species.

Gall Development in Plants Systemically Infected with *A. rubi*

The boysenberry and Himalaya blackberry plants used were systemically infected with *A. rubi*, and the galls which formed along the stems of the floral canes resulted not from wound infections but from the migration of the bacteria or a tumor-producing stimulus up the cane. Galls were never found occurring naturally on the primo canes, but appeared very frequently on the floral canes. Development of the galls began during the month of May and ceased during July. A cross section of the stem (Figs. 1, 8) shows that a thick cuticle covers the epidermis, which in turn surrounds the cortical region which consists of a more or less continuous belt of collenchyma, a slightly active phellogen, and a zone of thin-walled parenchyma. The pericycle is divided very distinctly into three layers. The outer layer forms a continuous sheath around the entire stele; the middle layer is composed of groups of thick-walled, lignified fibers, which stand out prominently over the vascular bundles (Fig. 7) and indicate whether excessive cell development has occurred interior or exterior to this zone. The cells in the inner layer are thin-walled and adjoin the phloem. The phloem and xylem tissue, which is composed of thick-walled fibers, tracheids, and vessels, is broken into bundles by the medullary rays. The rays are particularly prominent in the phloem region,

[1] *Manuscript received March 19, 1954.*
Contribution No. 1381 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] *Associate Plant Pathologist, Plant Pathology Laboratory, Saanichton, B.C.*

owing to the fact that strongly lignified tracheids are present. Apart from having a larger amount of xylem and phloem tissue, the floral cane appears to be quite similar to the primo cane.

Galls resulting from systemic infection of *A. rubi* may appear along the internodes as ridges and as beads at the nodes. In some instances the epidermis tears in the center of the gall (Fig. 2), and in others it may break at either side (Fig. 1). In Fig. 1, it will be observed that the stimulation may be restricted to a relatively narrow region and, after the gall breaks through the pericycle and cortex, it may fan out underneath the epidermis. In some instances, lateral pressure is sufficient to split the cane and cause drying and death to the terminal portion.

Organization within the galls frequently appears to be lacking, as is shown in Fig. 5 in which the metaxylem with scalariform pitting can be seen passing out at right angles from the cells of the extended medullary ray. Figs. 3 and 4 clearly show that excessive cell development occurs at or near the cambium, since radial rows of cells are continuous from the cambium to the outer limits of the gall. The tissues exterior to the middle pericycle are normal in size, and no abnormal amount of cell division has occurred in the outer pericycle. Frequently, crystals (Fig. 6) composed of calcium oxalate are found within the gall cells.

In Fig. 9, a cross section of a gall on the Himalaya blackberry is shown: the epidermis has been broken over the middle of the gall and thus covers only the edges of the gall. It may be observed that the thick-walled, lignified fibers of the middle pericycle have been pushed upward and to either side as the gall developed. This again definitely shows that gall development occurs in some tissue interior to the middle pericycle, and numerous observations indicate that rapid cell division occurs in the cambial region and particularly in the region where the medullary rays cross the cambium.

If the stem of a healthy floral or primo cane is cut into sections one-quarter or one-half inch in length and the sections are placed in petri plates containing a small amount of water, an abundant amount of growth is produced in the cambial zone (Fig. 10). If these sections are observed 24 hr. after they have been cut, it will be found that little lumps of growth form at the junction of the ray and the cambium. Growth continues until the small masses coalesce to form a complete circle. This tissue may continue to enlarge until it is one-half inch in height, which may be greater than the thickness of the section.

Vascularization of Plant Galls

As may be observed in Figs. 11 and 12, the vascular tissue in the gall on the sugar beet and the broad bean appears to have no developmental pattern. Also, several investigators (4, 5) working with plant tumors have claimed, after sectioning gall tissue, that the vascular tissue in the galls is completely unorganized and has no connection with the fibrovascular tissue in the stem or other organ upon which it may grow. It is doubtful, however, if these claims could be correct, because the galls may grow rapidly and become very

Fig. 1. A cross section of a gall on the floral cane of a boysenberry. Observe that tumorous tissue begins in the cambial region and passes out through the pericycle and cortex, and then spreads out under the epidermis and outer part of the cortex. Fig. 2. A cross section of a gall on the floral cane of a boysenberry, in which a large arc of host tissue is involved. The middle pericycle which stands out prominently over the bundles has been thrown outwards by the gall tissue.

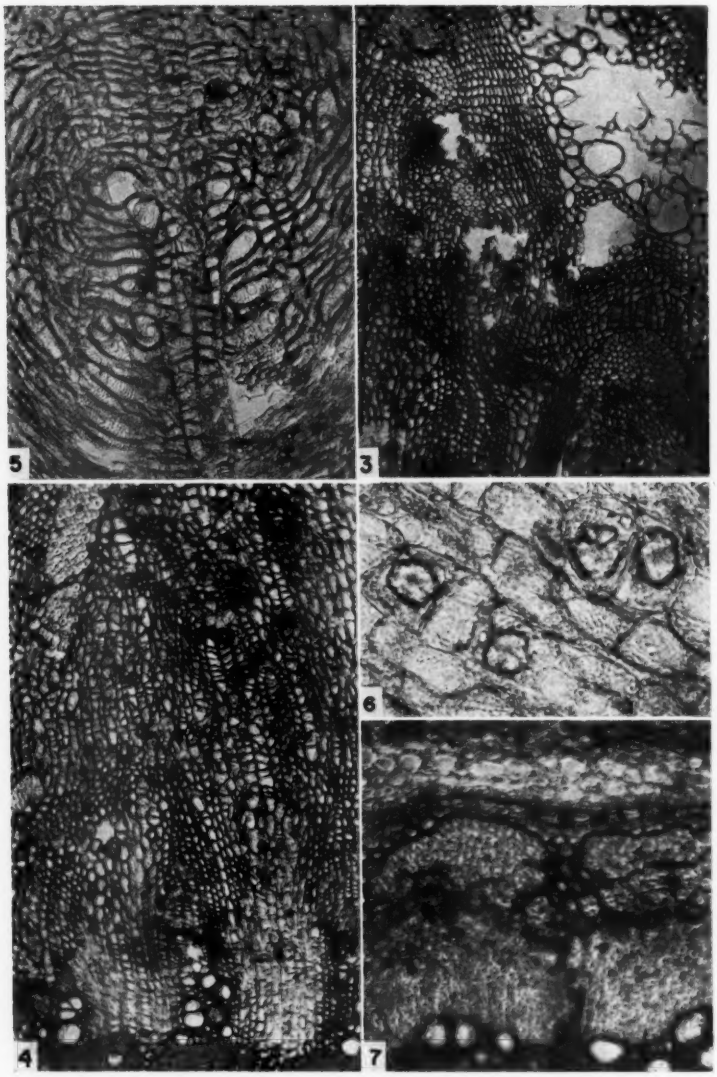


Fig. 3. An enlargement of the right side of Fig. 2; the middle pericycle which serves as a marker is no longer in the tangential plane but in the radial plane. Fig. 4. An enlargement of the left side of Fig. 2, which shows the radial arrangement of the cells in the gall. Fig. 5. A portion from the center of the gall, showing that tangential rows of scalariform cells may pass out from radial rows of cells. Fig. 6. This figure shows four calcium oxalate crystals within some gall cells. Fig. 7. A portion of a section of a floral cane. The continuous sheath of outer pericycle and thick-walled middle pericycle cells capping the bundles may be observed.

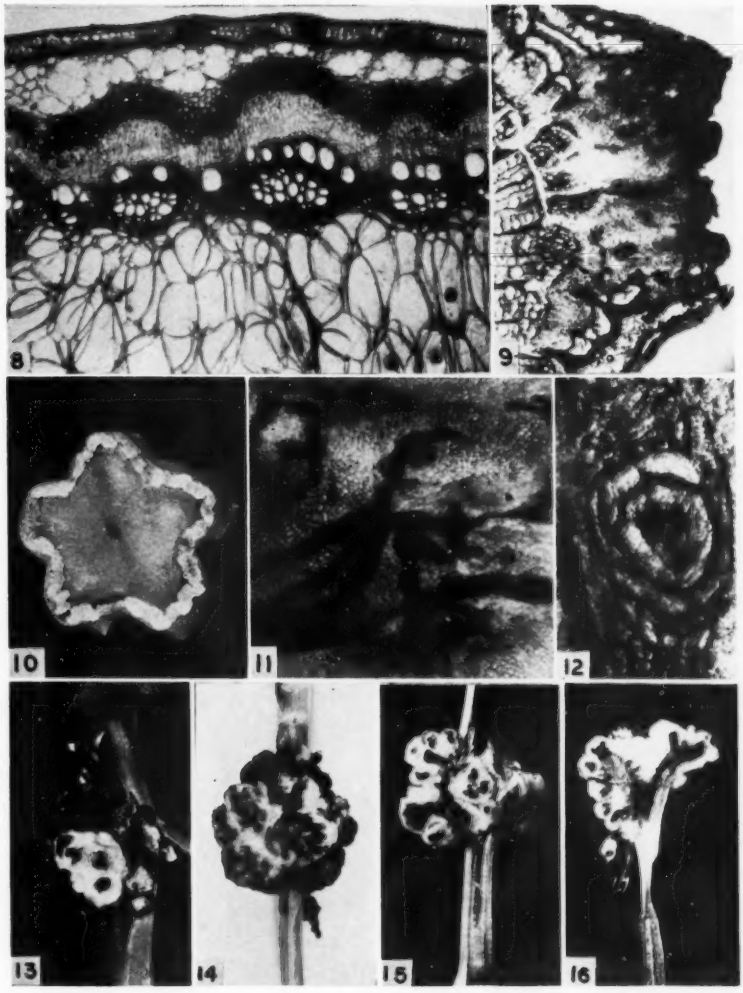


Fig. 8. A cross section of a primo cane, made in early June. Fig. 9. A cross section of a gall on the Himalaya blackberry. It may be noticed that development has taken place within the middle pericycle. Fig. 10. A ring of tissue on the cambium of a section of a floral cane from the Himalaya blackberry. Fig. 11. A section of a gall on broad bean. The black areas represent vascular tissue and thus display the irregular arrangement. Fig. 12. This figure shows the circular arrangement of some cells with scalariform pitting in sugar beet gall. Fig. 13. A broad bean stem with a gall which has been stained with acid fuchsin. A section has been cut off the surface of the gall, and the dark areas in the center of the gall are the red-stained vascular tissues. Fig. 14. Similar to Fig. 13, except that more of the gall has been cut away. Figs. 15 and 16. These figures show how the vascular tissue in the gall connects with that in the stem.

large in a relatively short time, and it is difficult to conceive of such rapid development occurring in the absence of continuous vascular tissue.

To test this theory, the basal cut ends of some broad bean, tomato, sugar beet, loganberry, and Himalaya blackberry plants, which contained large galls, were placed in a concentrated solution of acid fuchsin. In approximately two or three hours, the veins in the leaves were deeply stained and the vascular system in the stem could be readily distinguished from the other tissues. Sections of the gall showed that certain regions in the gall were stained (Figs. 13, 14) by this time also, and after examining them microscopically, it was obvious that only the vascular elements were stained. Longitudinal sections showed that the vascular strands of the stem passed out into the gall, and within the gall several vascular branches were produced. One branch always passed into each lobule or bulge on the gall, and the whole gall was completely vascularized. Since all of the vascular tissue is stained, even that which appears to be in isolated pockets when examined microscopically, there must be a connection between all vascular elements. The connection may be readily observed if a tumor which is stained with acid fuchsin is carefully dissected. Longitudinal sections show that the woody tissue of the stem fans out into the gall (Figs. 15, 16), and frequently bifurcates into the lobules that form on the galls.

Conclusion

In Jones' "Anatomical study of crown-gall tumours on the Himalaya giant blackberry (*Rubus procerus*)" (3), it is stated that the galls originate from the outer layers of the pericycle. This definitely is in conflict with the findings in this study, since the groups of fibers in the middle pericycle are always on the outside of the gall. However, he studied galls which he believed were the result of a wound infection with *Bacterium tumefaciens*, while the author used galls from the same plant and which resulted from a systemic infection of *Agrobacterium rubi*. The author is inclined to believe that the causal organism in both cases was the same species, that is, a strain of the bacterium commonly known as *A. rubi* in North America.

There seems to be no obvious anatomical reason why galls occur naturally on the floral canes and never on the primo canes. It is quite obvious that there is very little cambial activity in the stems of the floral canes as compared to that in the primo canes, but it has been demonstrated that the cambium may be stimulated into an active condition by bacteria or by wounding. However, perhaps it should be mentioned that galls occur more frequently at the nodes than along the internodes, and this may be due to a greater likelihood of injury or a greater activity of the cambium in this area, as shown by Brierly (1).

By the use of acid fuchsin, it has been clearly demonstrated that the vascular system in the galls on broad bean, tomato, sugar beet, loganberry, and Himalaya blackberry is connected to the vascular system of the organ upon which they are growing, even although observations on sections might indicate that the vascular system was completely unorganized.

Hildebrand (2) has stated that "Ordinarily the ultimate size of the gall was correlated with the depth of the wound but was independent of the size of the initial inoculum. The largest galls observed resulted from inoculating deep stem wounds, regardless of whether the inoculum was a single bacterium or a large number of bacteria." When deep inoculations occur, the vascular system of the organ is injured, and the gall which later forms has a good vascular system which is connected to the main system in the plant, and thus the size of the gall is only indirectly correlated with the depth of wounding.

References

1. BRIERLY, W. G. A study of senescence in the red raspberry cane. Univ. Minn. Agr. Expt. Sta. Tech. Bull. 69: 1-36. 1930.
2. HILDEBRAND, E. M. A micrurgical study of crown gall infection in tomato. J. Agr. Research, 65: 45-59. 1942.
3. JONES, S. G. An anatomical study of crown gall tumors on the Himalaya giant blackberry (*Rubus procerus*). Phytopathology, 37: 613-624. 1947.
4. RIKER, A. J. Some morphological responses of the host tissue to the crown gall organism. J. Agr. Research, 26: 425-435. 1923.
5. Von APPLER, H. Uber die tumorbildung durch *Pseudomonas tumefaciens* und durch heterauxin an *Helianthus annus*. Biol. Zentr. 70: 452-469. 1951.

CYTOLOGICAL STUDIES IN ALFALFA POLYPLOIDS[1]

By J. M. Armstrong[2]

Abstract

Normal tetraploid alfalfa, *Medicago sativa L.*, is characterized by meiotic irregularities consisting of a low percentage of univalents, trivalents, and quadrivalents. While these irregularities might suggest an autoploid origin, their frequency is too low to be conclusive. Cytological studies of the induced octoploid and of the hexaploid, obtained from crossing tetraploid and octoploid, indicate that the two genomes in the tetraploid are only partially homologous. The partial homology is established by the meiotic behavior in the hexaploid in which a low univalent frequency indicates fairly complete pairing between the *A* and *B* genomes. The comparatively low frequency of quadrivalents in the octoploid indicates a correspondingly low chiasma frequency at pachytene. Nevertheless this quadrivalent frequency in the octoploid is more than three times as high as in the tetraploid which suggests a lack of complete homology between the two genomes. The theory is advanced that tetraploid species of *Medicago* originated from crosses between a series of diploid species fairly similar cytologically but differing in well marked, morphological characters. This affords an explanation for the inheritance of some characters in a disomic and others in a tetrasomic manner. Cytological and genetic evidence thus points to tetraploid alfalfa as originating as an alloploid from closely related diploid species.

Introduction

In the genus *Medicago*, Darlington and Janaki Ammal (5) list 39 species whose chromosome numbers have been determined. These are either diploid or tetraploid with a basic chromosome number of seven or eight. The basic number eight appears to be the general rule since it occurs in 28 diploid and nine tetraploid species. Of the nine tetraploid species there are three, *M. sativa, L.*, *M. falcata L.*, and *M. lupulina L.*, in which occasional diploid plants have been found.

By far the most important species economically is *M. sativa*. A variant of this, usually given species rank as *M. media* Pers., is supposed to be a natural hybrid between *M. sativa* and *M. falcata*. There has been considerable speculation as to the origin of tetraploid *M. sativa*, whether by a process of auto- or alloploidy.

Early genetic studies showed predominantly disomic ratios in inheritance and it was generally assumed that *M. sativa* was an alloploid. More recently Stanford (11) reported a clear-cut case of tetrasomic inheritance in flower color. Twamley (13), in extensive studies of flower color inheritance, noted both disomic and tetrasomic inheritance patterns. One of the two factors associated with the production of purple pigment appeared to follow a tetrasomic exclusively whereas the second factor followed a disomic pattern in some plants and a tetrasomic in others.

[1] *Manuscript received December 31, 1953.*
Contribution from The Forage Crops Division, Central Experimental Farm, Ottawa, Canada.

[2] *Cytogeneticist, Forage Crops Division.*

Meiotic studies of the normal tetraploid have not been very extensive possibly owing to the difficulty of getting satisfactory preparations. Early work by Reeves (10) and Cooper (4) indicated the most frequent type of pairing at Metaphase I to be 16 bivalents. Recently Grun (7) made extensive studies of diakinesis cells from 12 plants and found an average of 0·62 quadrivalents per cell. The plant averages ranged from 0.21 to 0.89 quadrivalents per cell, the differences being statistically significant. In some cells as many as four quadrivalents were observed which is very suggestive of autoploidy. Univalents at diakinesis were also observed by Grun (7) to occur in 28% of the cells. The number per cell varied from one to eight and here also differences between plants were significant.

Ledingham (9) reported that in a cross between normal tetraploid *M. sativa* and a diploid form of *M. falcata* used as the female parent, the F_1 plants were variegated in flower color but instead of expected triploids ($2n=24$) the plants were all tetraploids. This result was considered to be due to nonreduction of the chromosome number in the *M. falcata* ovules. The chromosome pairing at first metaphase in the hybrids was found to be fairly regular. If the *M. falcata* chromosomes paired among themselves the *M. sativa* chromosomes must have paired autosyndetically.

Julén (8) crossed induced octoploids ($2n=64$) with the normal tetraploid and obtained hexaploids ($2n=48$). In 34 cells at first metaphase studied from two plants, 71% showed no univalents while the remainder showed one or two univalents. All pairing associations at first metaphase appeared to be by bivalents. If the tetraploid consisted of two dissimilar genomes incapable of pairing there should be a high frequency of univalents in the hexaploid. The regular pairing implies at least partial homology between the two genomes.

Diploid forms occur in several tetraploid species of *Medicago*. Bolton and Greenshields (3) reported such a form in *M. sativa* in an introduction received from the U.S.S.R. It resembled tetraploid *M. sativa* in general characteristics although smaller in size and of a decumbent growth habit. The diploid plants were highly self-sterile but readily intercrossed. Studies of the somatic chromosomes showed four chromosomes with satellites in the normal tetraploid and two similar chromosomes in the diploid. The authors considered this to be supporting evidence for the autotetraploid origin of *M. sativa*.

Materials and Methods

Two lots of tetraploid alfalfa were used, plants from the French variety, Du Puits, and wilt-resistant selections from the United States. Octoploid plants were obtained by treating the tetraploids with colchicine. The seedling stage was chosen for treatment, the tops of the plants being immersed in a 0.4% colchicine solution for 12 hr. The treated seedlings were then

washed and replanted. When they reached the flowering stage, the individual branches on each treated plant were examined for evidence of chromosome doubling. From the Du Puits lot, 14 plants were obtained which on the basis of pollen size had produced some octoploid branches. In the wilt-resistant lot, 16 such plants were secured. The normal and octoploid were then regrown from stem cuttings in order to eliminate mixaploid tissue. The chromosome numbers in the cuttings were verified by root-tip examination. Octoploid plants were found to be highly self-sterile but they intercrossed readily. Crosses were made between tetraploid and octoploid plants using the former as the male parent and a number of hexaploids were obtained.

The diploid *M. falcata* was obtained from an introduction received from the U.S.S.R. in 1935. Morphologically it resembles tetraploid *falcata* closely although it is smaller in its vegetative parts and is highly self-sterile. The diploid *M. sativa* was kindly supplied by Dr. Bolton, Saskatoon and was intercrossed seed from plants of the original introduction. Crosses were made between these two diploid forms and an F_1 population grown. Only two plants were obtained from attempted crosses between the diploid and tetraploid plants. The plants were from a diploid *M. falcata* × tetraploid *M. sativa* cross.

The racemes in the young bud stage were fixed in absolute acetic acid (3 : 1) in early June, 1952, from the field nursery. The fixed material was kept in a refrigerator four to six months before study. Buds were dissected in a drop of acetocarmine on the slide, the bud debris removed, and the anthers smeared with a scapel. The cover slip was placed on and the slide heated over a steam bath for 30 sec. and then firmly pressed between blotting paper. If microscope examination showed useful stages the slide was made semipermanent by ringing the slip with a mixture of certo, corn syrup, and acetocarmine (1 : 1 : 1). While alfalfa presents obstacles to the smear method by reason of the small size of the buds and anthers, such preparations were worth the extra effort as better distributions of chromosomes in the cells were obtained by using this method than would have been obtained if sectioning techniques had been followed.

Meiotic observations were made at diakinesis and first metaphase. In any given plant at least 10 cells were analyzed. Additional observations were made of various pollen stages.

Results and Observations

Table I lists the plants studied, their chromosome number, and the average chromosome association for each plant. An average has also been calculated for each polyploid type. As a parameter of meiotic regularity the last column gives the total percentage of associations as bivalents, quadrivalents, and hexavalents. These associations may be presumed to disjoin regularly at anaphase as compared to odd-numbered associations.

TABLE I

COMPARATIVE DATA ON MEIOTIC BEHAVIOR OF ALFALFA POLYPLOIDS

Plant No.	2n Chr. Number	Associations of chromosomes						
		I	II	III	IV	V	VI	II, IV, and VI
28–17	32	0.30	15.50	0.10	0.10			98.1
29–8	32	0.90	14.60	0.25	0.15	0.05	0.05	93.7
28–3	32	0.16	12.68	0.16	1.50			98.6
27–11–2	32		14.00		1.00			100.0
Av.		0.34	14.19	0.13	0.69			97.6
29–7–1	64	0.70	24.70	0.10	3.40			98.4
29–9	64	1.60	18.20		6.20		0.20	97.5
28–25	64	1.30	16.33		7.00		0.33	97.9
29–17–1	64	1.20	25.00	0.60	2.30		0.30	95.3
Av.		1.20	21.06	0.18	4.72		0.21	97.3
30–7–1	48	0.72	14.00	0.14	3.86		0.57	97.6
30–7–2	48	1.00	12.00		3.50		1.50	97.9
30–7–3	48	1.60	10.00	0.40	4.20		1.40	94.1
30–7–4	48	0.88	14.13	0.12	4.25		0.25	94.8
Av.		1.05	12.53	0.16	3.95		0.91	96.1
30–13–1	32	0.90	13.30	0.30	0.90			94.4
30–13–2	32	0.70	13.70	0.30	0.80			95.6
Av.		0.80	13.50	0.30	0.85			95.0
M. sativa	16		8.00					
M. falcata	16		8.00					
M. sativa × *M. falcata*	16		8.00					

Tetraploids

28-17

This was an F_1 plant of a cross between Wilt-resistant No. 30 and Du Puits No. 17. It proved to be fairly regular in its meiotic behavior. Pairing by bivalents was usual. In only one cell in 10 was a quadrivalent observed. Eighty-eight per cent of the cells from anaphase I to telophase II showed no irregularity. Irregularities consisted of one to two lagging univalents.

29-8

This was an F_1 plant of a cross between two Du Puits selections. A good preparation was obtained of cells at diakinesis. Of the 20 cells examined six showed normal pairing by bivalents; four cells showed a trivalent and a univalent with the remainder bivalents; two cells had two quadrivalents; two showed one quadrivalent and two univalents; four showed two univalents and 15 bivalents; one cell showed an association of five chromosomes and another an association of six. The last two cells suggest that

multiple configurations were due in part to segmental interchange in addition to associations due to a possible autoploid origin.

In Fig. 10, associations of 13 II, 1 IV, and 2 I are clearly apparent. The quadrivalent appears as a ring of four in which the chiasmata are well terminalized. The bivalents are mainly end-to-end and for the most part are not completely terminalized. In Fig. 11, the chromosome association is similar but the quadrivalent appears as a chain. One univalent lies near the quadrivalent and the other near the smaller nucleolus. Their difference in size suggests that they are heteromorphic and this may be a factor in pairing failure. Figs. 12 and 13 show two anaphases from this plant in which chromosome separation has been regular. Attention is drawn to the presence of chondriosomes in the cytoplasm that under certain staining conditions might be mistaken for lagging univalents. In Fig. 12 they are aligned on the equator of the cell while in Fig. 13 they are more widely dispersed.

28-3

This was an F_1 plant from a cross between Wilt-resistant No. 4 and Du Puits No. 6. This plant showed the highest number of quadrivalents at metaphase I of any tetraploid examined. Two cells were observed with four quadrivalents, or one half of the total chromosome number. Anaphases and telophases appeared quite regular showing that the quadrivalents had followed a balanced pattern at disjunction.

27-11-2

This was a Du Puits selection that had been selfed one generation. This plant was unique in that only one type of association was observed, 14 bivalents and one quadrivalent per cell. A good preparation of cells at prophase stages was obtained for this plant. Fig. 8 shows one such cell at late pachytene. In this cell 10 bivalents can be followed throughout their length. The remaining configurations are somewhat obscured by the nucleoli. There is a very close similarity in length in the paired bivalents. While it is not possible to distinguish the chiasmata clearly at this stage there are noticeable regions where there is definite absence of pairing exchanges. Fig. 9 shows a portion of a cell at pachytene with three free ends. Two of these ends show an unpaired condition suggesting that these bivalents are non-homologous at their distal ends.

The four plants studied in the tetraploid group all display some departure from regular pairing by bivalents. The frequency of chromosome associations that are likely to produce abnormal tetrads are not high. Even the plant characterized by the most irregular behavior, 29-8, could produce over 90% normal pollen. The differences in average meiotic behavior among the plants can scarcely be considered as significant. While all plants show a variable frequency of quadrivalents this frequency cannot be considered high enough to prove that alfalfa is an autotetraploid.

Octoploids

Octoploid branches on treated plants were identified on the basis of pollen size, octoploid pollen being about 50 μ and tetraploid pollen 35 μ in diameter. A comparison of pollen is shown in Fig. 19. Octoploid plants free of mixaploid tissue were obtained by stem cuttings. These were then intercrossed and the plants studied cytologically in this group are from the first generation of these crosses. The plants examined were picked at random from several hundred octoploid plants.

29-7-1

This was an F_1 plant from two Du Puits octoploids. This plant showed a relatively low frequency of quadrivalents with no hexavalents observed. Only 22.0% of the chromosomes were associatied as multivalents, the associations being usually bivalents. Another observation was the low frequency of univalents. In six of the 10 cells analyzed univalents were absent and the other four cells showed only one or two. Fig. 14 shows a photograph of a cell at diakinesis, with 24 II and 4 IV. An interpretation of this photograph is given in Fig. 1. Fig. 16 shows a late anaphase in which two univalents have completed division.

29-9

This is also an F_1 plant from two Du Puits octoploids but of different parentage from 29-7-1. In this plant a higher frequency of quadrivalents was observed and an occasional hexavalent. Figs. 2 and 15 show a cell containing two hexavalents and one quadrivalent with the remaining chromosomes associated as bivalents.

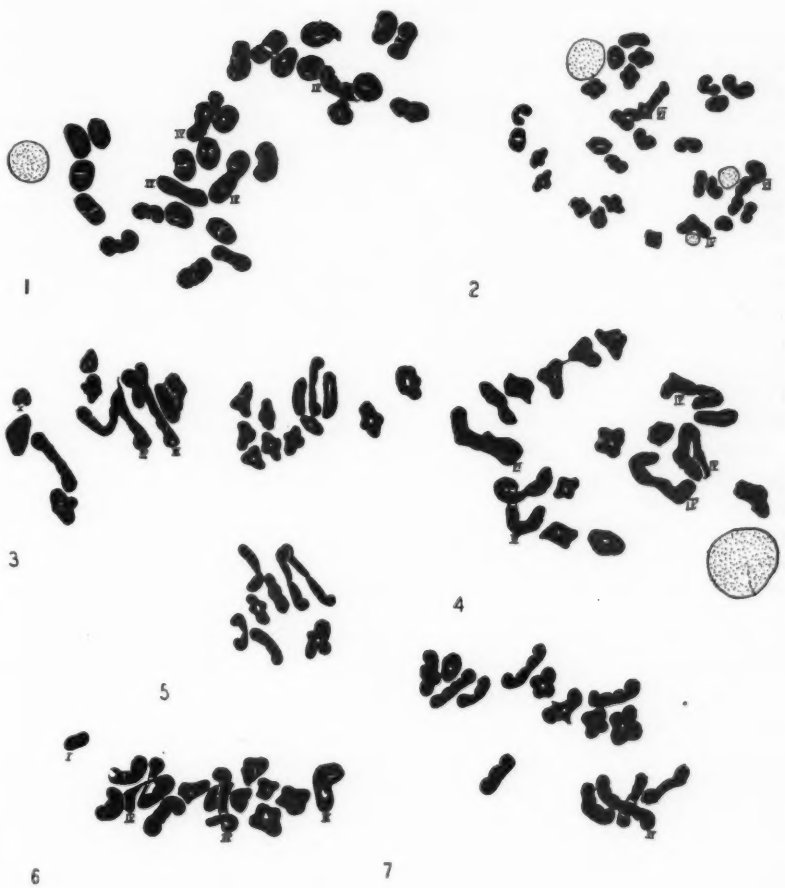
28-25

This is an F_1 plant from a cross between Du Puits and Wilt-resistant octoploids. Its meiotic behavior is very similar to that of the previous plant but with a slightly higher frequency of multivalents.

29-17-1

This is an F_1 plant from a cross of Du Puits octoploids. It resembles the first plant closely in the low frequency of multivalents.

The four octoploid plants studied cytologically show a range of variability in chromosome associations at diakinesis and first metaphase very similar to that observed in the tetraploids. This variability is very likely due to the differences in the parent tetraploid plants. Regardless of whether the tetraploid owes its origin to auto- or alloploidy, the octoploid contains chromosomes that are homologous in sets of four, and it might be logical to expect a high frequency of quadrivalents. Since this frequency is rather low it can be inferred that the tendency for chromosome association by bivalents is due to conditions at pachytene. If chiasma frequency is low, there is less opportunity for multiple chromosome associations.



Figures 1–7 are camera lucida drawings which have been reproduced at a magnification of approximately × 1700.

FIG. 1. Diakinesis is an octoploid showing four quadrivalents and 24 bivalents. This is a camera lucida drawing of Fig. 14. FIG. 2. Diakinesis in an octoploid showing two hexavalents, one quadrivalent, and 24 bivalents. This drawing is similar to Fig. 15. FIG. 3. Metaphase I of hexaploid corresponding to photograph in Fig. 17. The chromosome associations consist of one quadrivalent, one trivalent, 20 bivalents, and one univalent. FIG. 4. Diakinesis of hexaploid showing two hexavalents, three quadrivalents, and 12 bivalents. FIG. 5. Metaphase I of F_1 plant from diploid *M. falcata* × diploid *M. sativa* showing types of bivalent configurations and varying degrees of chiasmata terminalization. FIG. 6. Metaphase I of induced tetraploid showing two quadrivalents, one trivalent, 10 bivalents, and one univalent. FIG. 7. Metaphase I of induced tetraploid corresponding to photograph in Fig. 18. The chromosome associations consist of 14 bivalents and one quadrivalent.

Hexaploids

The hexaploids were obtained by crossing tetraploids with octoploids using the tetraploid as the pollen parent. Crossing in this manner was readily carried out since all the octoploids proved to be self-sterile. The four plants studied cytologically have the same female parent, Wilt-resistant No. 34, but the male parents were different in all cases. The hexaploids proved to be more vigorous than the octoploids, exhibiting more profuse tillering.

The cytological behavior of the four plants listed in Table I is essentially similar. There is a very low frequency of univalents which is in agreement with the observations of Julén (8). The writer's observations in regard to multivalents are somewhat at variance with those of Julén's since he reported only pairing by bivalents while the multivalent frequency in the writer's material was relatively high. No cells were free of at least one quadrivalent and occasionally the frequency was as high as five. In addition hexavalents were observed. The multivalent frequency proved to be higher in the hexaploids than in the octoploids. On the average 32.3% of the chromosomes were in multiple associations in the octoploids and 45.3% in the hexaploids.

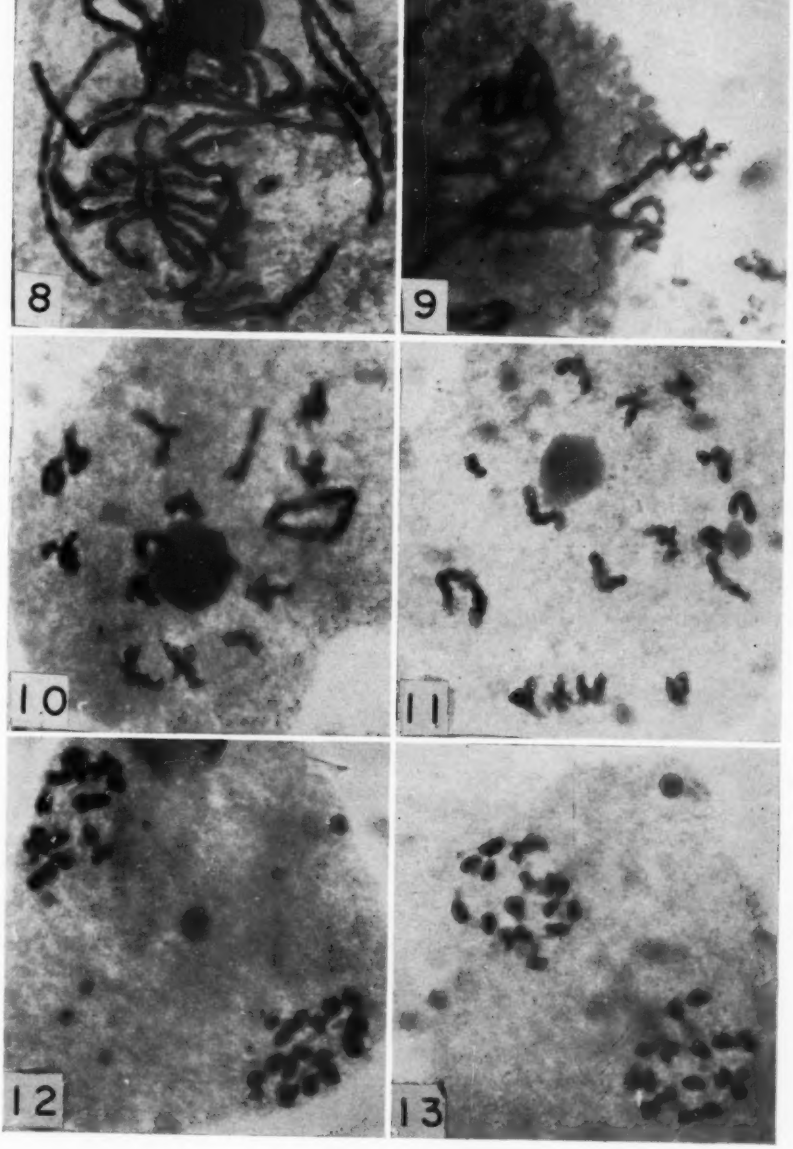
Figs. 3 and 17 show a cell with the nearest approach to pairing by bivalents. In this cell 1 IV, 1 III, 20 II, and 1 II may be observed. Fig. 4 shows the opposite extreme of a high number of multiple configurations. In this cell there are 2 VI, 2 IV, and 12 II. Occasionally at diakinesis two bivalents may show a slight matrix adhesion as at 11 o'clock in Fig. 4. Such configurations are not considered to be quadrivalents as this matrix attachment is not a true chiasma.

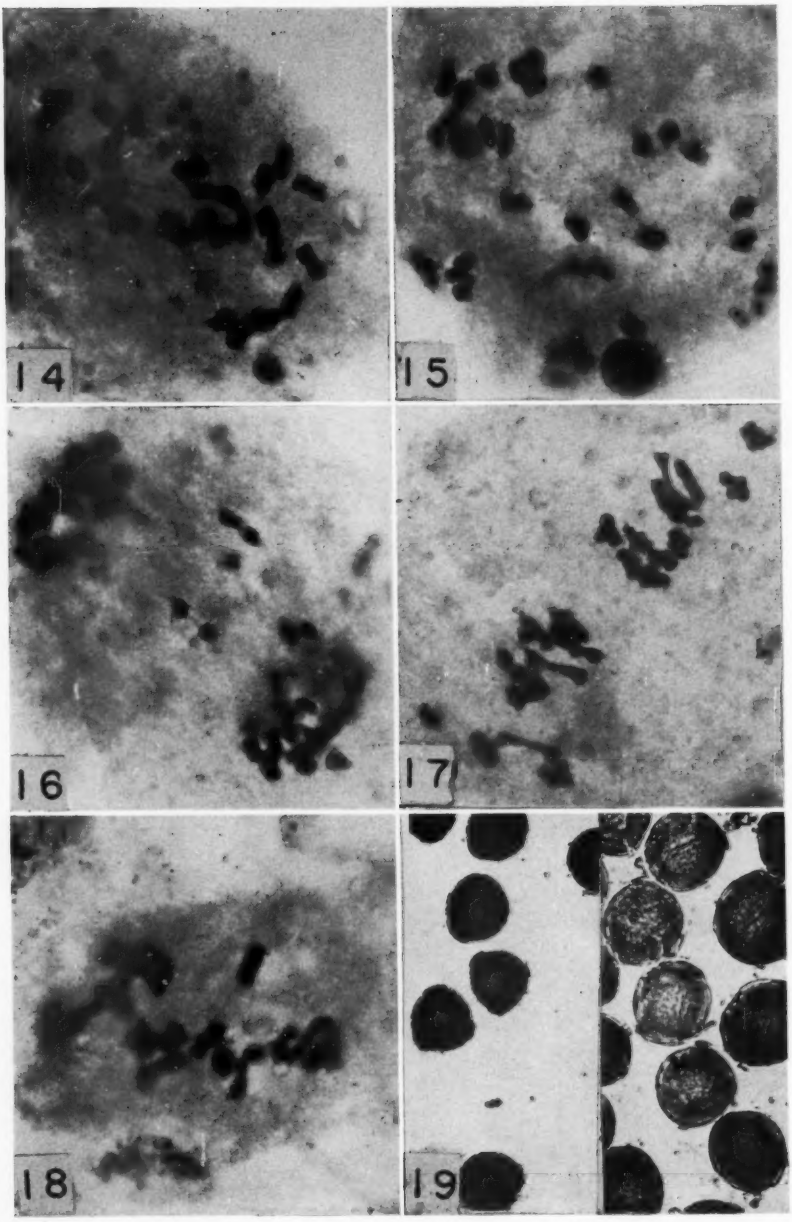
Diploids

A few plants of the diploid forms of *M. sativa* and *M. falcata* were examined. They proved to have quite normal meiosis in which eight bivalents were regularly present at first metaphase. Crosses were made between the two forms and F_1 plants which were examined likewise showed quite normal meiotic behavior. Fig. 5 shows a typical cell at first metaphase. Seven of the bivalents have single chiasmata, in five of which terminalization is complete. The remaining bivalent appears to be a ring with two terminalized chiasmata. The configurations are suggestive of a low initial chiasma frequency at pachytene.

Figures 8–13 are photomicrographs which have been reproduced at a magnification of approximately × 1700.

FIG. 8. Early diplotene in tetraploid showing chromatids starting to open into loops. This distribution of chiasmata differs markedly in different bivalents. FIG. 9. Late pachytene in tetraploid showing pairing completed at one terminal end and incomplete at two ends. FIG. 10. Diakinesis in tetraploid showing a ring quadrivalent, two univalents and 12 bivalents. FIG. 11. The chromosome associations are similar to Fig. 10 except that the chromosomes in quadrivalent are end-to-end. The univalents appear to be heteromorphic. FIG. 12. Normal telophase in tetraploid; chondriosomes may be observed at equator of cell. FIG. 13. Similar to Fig. 12 except that the chondriosomes are distributed at random.


8
9
10
11
12
13


14
15
16
17
18
19

Tetraploid Hybrids

In an effort to obtain triploids, tetraploid *M. sativa* plants were crossed with diploid *M. falcata*. No seed was obtained using the diploid as the male parent. In the reciprocal cross from 16 crossed florets two seeds were obtained where normally 50–75 seeds may be expected from compatible parents. The resulting F_1 plants were variegated in flower color attesting to the presence of factors from both parents. Somatic counts of the chromosomes from the root tips showed that the plants were tetraploids. In a similar cross Ledingham (9) obtained only tetraploids. He suggests that the tetraploids were due to the fertilization of unreduced female gametes by normal pollen. The lack of triploids from this cross indicates a failure of fertilization of normal gametes or the early abortion of the resulting embryos.

The results of the cytological examination of these two tetraploids are given in Table I. The average chromosome associations are in general similar to those of normal tetraploid plants. There is however a somewhat higher frequency of univalents and quadrivalents. It seems reasonable to assume that the 16 *falcata* chromosomes form eight bivalents and hence the two sets of chromosomes received from the *sativa* parent must also pair autosyndetically. The occurrence of quadrivalents indicates that the two sets are partly homologous and interpair to some degree. Fig. 6 shows a first metaphase of plant 30–13–2 with 2 IV, 1 III, 10 II, and Figs. 7 and 18 show a cell with 14 II and 1 IV.

Discussion

The cytological evidence submitted here throws some light on the possible origin of tetraploid alfalfa. It seems clear that the two genomes in the tetraploid possess in common a considerable number of homologous chromosome sections or even whole chromosomes. This homology is extensive enough to permit fairly complete pairing between chromosomes of the two genomes. This is evident from the meiotic behavior in the plants derived from the diploid *falcata* × *tetraploid sativa* cross. It is reasonable to assume that the chromosomes derived from the unreduced *falcata* gamete pair normally. It would follow that the single genomes derived from the *sativa* gamete must pair autosyndetically. This pairing is not quite as good as in normal *sativa* since the frequency of bivalents is somewhat lower and other configurations of univalents, trivalents, and quadrivalents correspondingly higher. There

---

Figures 14–19 are photomicrographs. Figs. 14–18 have been reproduced at a magnification of approximately × 1700. Fig. 19 was reproduced at × 280.

FIG. 14. Late diakinesis in octoploid showing four quadrivalents and 16 bivalents. FIG. 15. Diakinesis in octoploid showing two hexavalents one quadrivalent and 16 bivalents. FIG. 16. Late anaphase I in octoploid showing two univalents at equator that have just completed the homotypic division. FIG. 17. Metaphase one of hexaploid showing one quadrivalent, one trivalent, 20 bivalents, and one univalent. FIG. 18. Metaphase I of induced tetraploid showing 14 bivalents and one quadrivalent. FIG. 19. Pollen sample of tetraploid (left) and octoploid (right). The former averages 35 and the latter 50 μ in diameter. × 280.

is evidently a preferential affinity for chromosome pairing within the normal tetraploid. The homologous chromosomes in the same genomes pair more readily than partly homologous chromosomes from separate genomes.

The hexaploid supplies much the same line of evidence. If we assign the genomic formula *AABB* to the tetraploid, then the octoploid and hexaploid will be *AAAABBBB* and *AAABBB* respectively. In the hexaploid preferential affinity would lead to pairing in the genomes *AA* and *BB*. It is apparent from the low frequency of univalents that pairing is extensive between the remaining *A* and *B* groups of chromosomes.

The study of the octoploid has an important bearing on the problem since it supplies information on chiasma frequency. Disregarding the suggested partial homology between the genomes, the constitution of the octoploid is such that it contains homologous chromosomes in sets of four permitting maximum opportunity for quadrivalent formation. The average quadrivalent formation was observed to be 4.72 per cell or 29.5% of the possible maximum. Numerous autotetraploids have been produced in recent years and the quadrivalent frequency has been observed to vary widely. Armstrong (1) in an annual rape tetraploid found that 55% of the chromosomes at first metaphase were in quadrivalent associations. On the other hand quadrivalent frequency may be very low as in the induced tetraploid of *Plantago maritima L.* reported by Earnshaw (6). Alfalfa would appear to fall in the latter category although in it the frequency may not be extremely low. Quadrivalent formation is probably determined by the initial chiasma frequency at pachytene. If chiasma frequency is low the chromosome associations would tend to be more by bivalents rather than by quadrivalents in the octoploid.

A low frequency of hexavalents was observed in the octoploid. This averaged 0.21 per cell or 1.97% of the total chromosome complement. This occurrence of hexavalents parallels quadrivalent association in the tetraploid and is presumably due to associations of chromosomes from the *A* and *B* genomes. A low chiasma frequency would be a limiting factor of multiple chromosome configurations due to crossing over interference. The more complex associations would be less frequent. The observed frequencies bear out this expectation fairly well since quadrivalent associations in the tetraploid comprised 8.62% of the total chromosome complement as compared to 1.92% hexavalents in the octoploid.

The comparison of quadrivalent frequencies in the octoploid and tetraploid leads to another conclusion. If it is assumed that *M. sativa* is an autotetraploid with limited quadrivalent formation because of low chiasma frequency, there appears to be no reason why this frequency should not be of the same magnitude as in the octoploid. However the frequencies are 8.61 and 29.5% for tetraploid and octoploid respectively. This might imply that another factor limits quadrivalent formation in the tetraploid and it appears logical to assume that this factor is the lack of complete homology between the *A* and *B* genomes.

The above conclusions on low chiasma frequency and lack of complete homology between the *A* and *B* genomes is supported by direct observations of prophase. Extensive chromosome regions at pachytene and early diplotene in cells of the tetraploid were observed where there was an absence of pairing exchanges. This suggests contiguous chromosome segments lacking homology. The flaring chromosome ends in Fig. 9 also suggests structural differences in chromosomes. In some cells at diakinesis where two univalents were present these univalents were observed to differ in size as in Fig. 11. These may be heteromorphic homologues but are more probably chromosomes from separate genomes whose partners have paired owing to partial homology. This explanation would imply that some chromosomes in the *A* and *B* genomes have major structural differences. Observations of the bivalents in cells at diakinesis as in Figs. 10 and 11 show that many bivalents are associated by a single chiasma as yet unterminalized. This is very suggestive of a low initial chiasma frequency.

Attention was drawn in the literature review to the considerable number of characters whose inheritance is disomic in type. Atwood and Grun (2) in summarizing these investigations listed 36 studies covering 25 characters. Where genetic interpretations have been proposed, they are on the basis of disomic ratios. Two recent investigations were cited (11, 13) where the authors explain the results on the basis of tetrasomic inheritance. The crossability of some *Medicago* species and the fertility of the resulting hybrids has been demonstrated for several species such as *sativa*, *falcata*, *glutinosa*, and *gaetula*. The hybrids that have been investigated cytologically show fairly normal meiotic behavior. Yet these species have been rated as such on the basis of many distinct morphological characters. If we assumed a series of diploid species fairly similar cytologically yet differing by well-marked morphological characters, hybridization of such species followed by chromosome doubling would yield a series of tetraploid species. Characters common to the two parent species might be expected to be inherited in a tetrasomic manner while distinctive characters would be disomic in type. The present scarcity of demonstrably tetrasomic characters exists possibly because many of these characters are quantitative.

Not only does the *Medicago* genus contain many diploid species to permit opportunity for the hybridization which the above hypothesis requires, but several tetraploid species contain diploid forms. Two such forms in *M. sativa* and *M. falcata* were included in the present study. It is unlikely that they represent reversions from tetraploid to diploid as they were reputably collected from areas where wild-growing plants were prevalent. They are also likely to be species hybrids since they conform to the description of segmental alloploids as described by Stebbins (12). They show normal meiotic behavior and display almost complete self-sterility with a low degree of cross-fertility. The induced chromosome doubling of these diploids and a comparison with existing tetraploids should prove a valuable line of investigation.

References

1. ARMSTRONG, J. M. A tetraploid form of annual rape induced by colchicine. Trans. Roy. Soc. Can. 44 : 21-38. 1950.
2. ATWOOD, S. S. and GRUN, P. Cytogenetics of alfalfa. Bibliographia Genetics, 14: 133-188. 1951.
3. BOLTON, J. R. and GREENSHIELDS, J. E. R. A diploid form of *Medicago sativa* L. Science, 112 : 275-277. 1950.
4. COOPER, D. C. Macrosporogenesis and embryology of *Medicago*. J. Agr. Research, 51 : 471-477. 1935.
5. DARLINGTON, C.D. and JANAKI AMMAL, E. K. Chromosome atlas of cultivated plants. Univ. Press, Aberdeen. 1945.
6. EARNSHAW, F. Experimental taxonomy. V. Cytological studies in sea plantains allies to *Plantago maritima* L. New Phytologist, 41 : 151-164. 1942.
7. GRUN, P. Variation in the meiosis of alfalfa. Am. J. Botany, 38 : 475-482. 1951.
8. JULÉN, G. Investigations on diploid, triploid and tetraploid lucerne. Hereditas, 30 : 567-582. 1944.
9. LEDINGHAM, G. F. Cytological and developmental studies of hybrids between *Medicago sativa* and a diploid form of *M. Falcata*. Genetics, 25 : 1-15. 1940.
10. REEVES, R. G. Nuclear and cytoplasmic division in the microsporogenesis of alfalfa. Am. J. Botany, 17 : 29-60. 1930.
11. STANFORD, E. H. Tetrasomic inheritance in alfalfa. Agron. J. 43 : 222-225. 1951.
12. STEBBINS, G. L., JR. Variation and evolution in plants. Columbia Univ. Press, New York. 1950.
13. TWAMLEY, B. E. Flower color inheritance in diploid and tetraploid alfalfa. Ph.D. Thesis, University of Saskatchewan, Saskatoon, Sask. 1952.

A HITHERTO UNREPORTED ASCOMYCETE PARASITIC ON THE ROOTS OF WHEAT, OATS, AND BARLEY[1]

By D. B. Robinson[2] and G. W. Ayers[2]

Abstract

An ascomycetous fungus, named *Cryptoascus graminis* sp. nov., is described. This fungus is found parasitic on the roots of wheat, oats, and barley in Prince Edward Island.

A soil-inhabiting ascomycete has been observed annually since 1937 on the roots of cereal crops in Prince Edward Island. The fungus is found chiefly on the roots of wheat and occurs occasionally on the roots of oats and barley. Infection is confined to epidermal tissue and is greatest on young rootlets, so that the fungus is always more abundant on the coronal than on the seminal root system (Fig. 1). The host plant apparently suffers little injury even when the parasite is very abundant.

The stromata and perithecia are gelatinous during early development but become brittle and carbonaceous with maturity. Perithecia are irregularly oblong to ovoid and are borne in clusters, commonly four or five in number, on a limited, black stroma. These clusters may be observed on the rootlets of the host in midsummer as minute, black bodies ranging up to a millimeter in diameter. Examination of crushed mounts of mature perithecia and of sections through perithecial clusters shows numerous asci measuring 50–60 × 35–45 μ, each containing two ascospores. The asci evanesce very early and so are usually not released into the surrounding medium. Ascospores are dark, granular or vacuolate, and fall within the range 35–42 × 15–18 μ.

No conidial state of the fungus has been observed.

This fungus is very similar to *Cryptoascus oligosporus* Petri, reported as occurring on the roots of olive in Italy (1). *Cryptoascus* has been named as a monotypic genus, but the fungus described in this paper appears to be congeneric in every way with Petri's species except that the asci are two-spored whereas *C. oligosporus* is described as monosporous. This character is not generally regarded as worthy of generic distinction in itself, so it is proposed that the generic diagnosis of *Cryptoascus* be emended by the deletion of the word 'monospori' and that the fungus herein described be named *Cryptoascus graminis* sp. nov.

Cryptoascus graminis sp. nov.

Peritheciis irregulariter oblongatis vel usque ovoideis, nigris, carbonaceis, basi hyphis nigris instructis, pseudoparenchymaticis, 125–225 × 80–100 μ, ascis globosis, gelatinosis, hyalinis, 50–60 × 35–45 μ, mox cutis evanescentibus, bisporatis; sporidiis ovoideis, granulosis vel vacuolatis, fuligineis, 35–42 × 15–18 μ.

[1] *Manuscript received April 6, 1954.*
Contribution No. 1383 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] *Associate Plant Pathologist, Plant Pathology Laboratory, Charlottetown, P.E.I.*

Hab. in radicibus Tritici aestivi, Avenae sativae et Hordei vulgaris.

Perithecia irregularly oblong to ovoid, black, carbonaceous, resting on a black stroma, pseudoparenchymatous, 125–225 × 80–100 μ; *asci* globular, gelatinous, hyaline, 50–60 × 35–45 μ, each containing two ascospores, walls evanescent; *spores* oval, granular or vacuolate, dark, 35–42 × 15–18 μ.

Found on the roots of wheat, oats, and barley.

Type obtained from Acadia wheat, Charlottetown Royalty, August, 1953, and deposited in the Department of Agriculture Mycological Herbarium, Ottawa, (DAOM 39448).

Reference

1. PETRI, L. Osservazioni sopra alcune malattie dell 'Olivo. Rend. accad. nazl. Lincei, 18 (2): 635–642. 1909.

(NOTE: *Plates I and II follow.*)

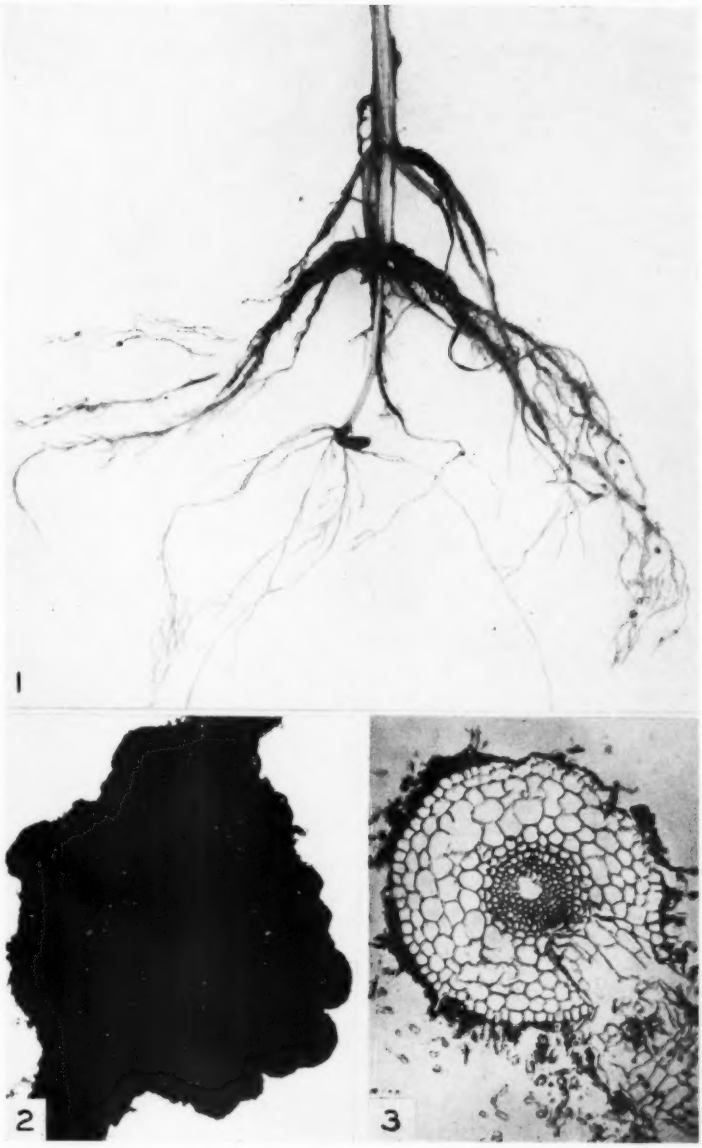


FIG. 1. Roots of Acadia wheat plant showing perithecial clusters. FIG. 2. A mature perithecial cluster. 50×. FIG. 3. Cross section of root showing fungus on epidermis. 90×.

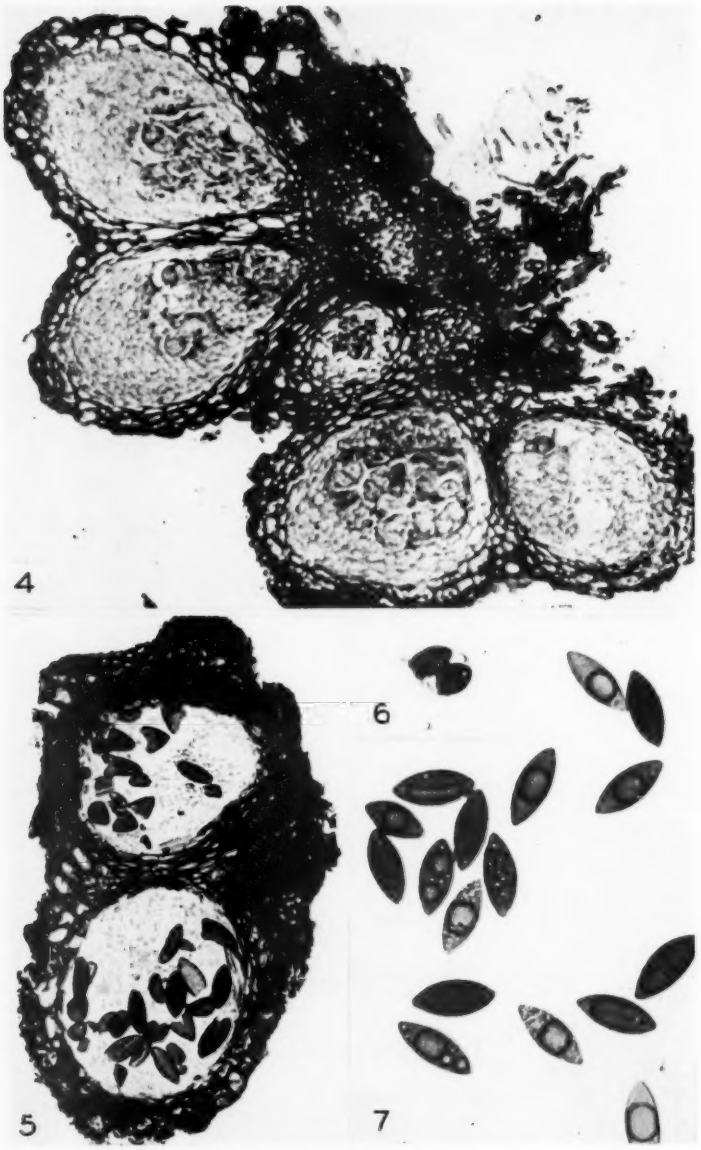


FIG. 4. Section through perithecial cluster showing developing asci. 220×. FIG. 5. Section through perithecia showing mature ascospores. 190×. FIG 6. Ascus containing two ascospores. 180×. FIG. 7. Ascospores. 340×.

FRUCTIFICATION OF *PORIA COCOS* (SCHW.) WOLF ON POPLAR[1]

By Clara W. Fritz[2]

Abstract

The subterranean sclerotia of *Poria cocos* (Schw.) Wolf have been known in North America for more than one hundred and fifty years, but sporophores of the fungus are rare. This paper reports the first fruit bodies of this fungus found in Canada. They developed on poplar pulpwood bolts during storage; the history of the logs from the time of cutting up to the finding of the sporophores is recorded.

The sclerotial stage of the fungus discussed in this paper has been known in North America since the latter part of the 18th century; but it was not until 1922 that Wolf (4) obtained a fruit body from one of these resting bodies, and found that the fungus belonged to the genus *Poria*. He named it *Poria cocos* (Schw.) comb. nov. Fruit bodies of the fungus must be rare in nature or of a very ephemeral character, since, in spite of the extensive host and geographical range which this *Poria* is known to cover, there are comparatively few records of the finding of sporophores. The present paper reports what are believed to be the first fruit bodies found in Canada.

Distribution

For over one hundred and fifty years records of the distribution of *P. cocos* were based on reports of the subterranean sclerotia from widely scattered localities in the United States of America. In 1929, Weber (3) listed collections reported by various mycologists from Delaware, New Jersey, New York, Pennsylvania, Virginia, Maryland, North and South Carolina, Tennessee, Georgia, Florida, Mississippi, Kansas, and Texas; he noted also records of its association with roots of cedar, citrus trees, corn, eucalyptus, fir, magnolia, oak, pine, and sumac. He stated also that, "on the outer coat of a number of sclerotia collected in Florida were found the remains of numerous clusters of weathered and somewhat disintegrated sporophores resembling fruiting structures of a *Poria*".

In 1942 Davidson *et al.* (1) described the cultural characteristics of the fungus and thus provided another aid for studying its distribution. He stated that identification "of a number of isolates from oak and other hosts" was made by comparison of these with a culture from a sclerotium collected in Maryland in 1935.

Canadian Culture Records

Poria cocos was first identified in culture in Canada by Dr. M. K. Nobles, Department of Agriculture, Ottawa (unpublished records). It has probably been isolated from decayed wood fairly frequently; but, since its mycelial

[1] *Manuscript received April 12, 1954.*

[2] *Formerly Timber Pathologist, Forest Products Laboratory, Ottawa, Canada.*

characteristics in culture differ from those usually associated with the wood-rotting Basidiomycetes, its importance in causing decay has been overlooked.

The first published record of *P. cocos* in Canada is that of Redmond and Robinson (2), the identification having been made by Dr. Nobles. They isolated the fungus from a butt rot of yellow birch (*Betula lutea* Michx. f.) in eastern Nova Scotia, and considered it the causal agent of the rot. They also studied its effect on the growth of yellow birch seedlings, and found that it rapidly proved lethal; its parasitic action on yellow birch was thus established.

During a study of rots of poplar pulpwood started in 1950 and just completed, the writer has made fairly frequent isolations of *P. cocos*. Identification of the isolates was made by comparison with the description of Davidson *et al.* (1) and with cultures supplied by Dr. Nobles, with which they agreed in all respects.

The pulpwood under study was cut on Manitoulin Island, Ontario, in the winters of 1951 and 1952. Of upwards of two hundred pulpwood bolts from which cultures were made, *P. cocos* was obtained from twenty-seven. It was isolated from butt logs and from rot associated with wounds in bolts from farther up the trunk. The fungus was active in the stored wood, and evidence was obtained which suggested that under some conditions it would spread from log to log in a pile. A discussion of storage conditions and the activity of the fungus in the several bolts will be given in an article to be published later; the present report deals only with the history of the two bolts on which fructification occurred.

Sporophore Production

Sporophores developed on two of the 208 logs under study, *logs No. 40 and 1970;* they were found when a final examination of the bolts was made in October 1953 and January 1954 respectively.

Log No. 40 was cut on Manitoulin Island in January 1952 and included in a pile of 86 logs which was established on the island. The bolts were stored unbarked in a corded pile and left undisturbed for one summer season. Late in the autumn the pile was dismantled; a section 1 ft. in length was cut from one end of each log and the remaining portions were shipped to Ottawa. On arrival they were piled in the open and covered with tarpaulins; a few weeks later they were removed to a well-ventilated storage shed so that they would be more readily accessible for study during the winter.

The work was interrupted, and it was not until May 1953 that *log No. 40* was reached for study. It was a large log, 11 in. in diameter; it contained large knots and an irregular core of yellow-brown rot; *Stereum purpureum* had fruited on the uncut end. A disc was cut from this end and the remaining portion was sawn into three sections approximately equal in length; the pieces were numbered 1–4 in order from disc to the other end.

Sections No. 2 and 3 were ripped and 37 cultures were made from wood thus exposed. The sections were then returned to the shed and piled on top of one another in upright position in order 4, 1, 2, 3; owing to further

interruption of the work the sections remained undisturbed until October 1953. In the meantime, however, the cultures had been examined and a record made of the fungi isolated.

In October 1953 the sections were removed from the shed and examined with a view to correlating the fungi obtained in culture with the rots in the wood. It was found that of 21 inocula taken from the yellow-brown core, 20 had yielded *P. cocos; Stereum purpureum* and *Polyporus hirsutus* had been obtained from the sapwood; two cultures had contained Hyphomycetes only.

P. cocos had been very aggressive during storage in the shed. It had produced thick, strong mycelial sheets between all the internal surfaces, transverse and longitudinal, which were in contact, thus locking the pieces together; on the bottom of section 4, which was resting on the floor, a very tough leathery felt had developed. The fungus had also penetrated from the yellow-brown core into the wood from which in May only *Stereum purpureum* had been isolated; it had produced the typical *P. cocos* rot in the wood invaded, and 12 inocula taken from this area yielded that fungus in pure culture.

Around the base of section 4 at the floor level *P. cocos* had fruited as shown in Fig. 1; sporophores encircled the section irregularly. Fig. 2 shows an enlargement of a portion of the fruit body seen in front view in Fig. 1. Cultures made from the tissue of three of the fructifications and from adjacent wood yielded *P. cocos*. The sporophores were sterile, but the cultures produced fertile surfaces from which spores typical of *P. cocos* were obtained.

Log No. 1970 was cut on Manitoulin Island in January 1951 and was included in a pile of 76 bolts which was established in a sheltered side on the island. The bolts were stored unbarked in a corded pile as in the case of the pile containing *log No. 40* discussed above. The pile remained undisturbed until late autumn 1952, thus passing two summer seasons in field storage; it was then dismantled. Subsequent treatment of the logs was the same as that described for *log No. 40*.

Log No. 1970 was examined and cut into sections in March 1953. It was 10 to 11 in. in diameter and contained a large grayish core with a yellowish central area in which there was some reddened wood; the rot was uniform through the bolt and resembled that caused by *Radulum casearium; Stereum purpureum* had fruited on the end and side. Sections 2 and 3 were ripped, and 40 cultures were made from the wood exposed. The sections were then piled in the shed in order 4, 1, 2, 3; they were not reached for further study until January 1954.

The original core was still readily recognizable, but a vigorous invasion of *P. cocos* had taken place during the summer. The cultures made in the spring had not yielded a primary wood-rotting fungus; only Hyphomycetes had been isolated. From beyond the core the storage-rot fungi, *Stereum purpureum*, *Polyporus adustus*, and *Corticium laeve* had been obtained in addition to Hyphomycetes. In January, thick felts of *P. cocos* were found: a very strong, leathery felt on the bottom of section No. 4 in contact with the floor,

thick but softer felts binding together the sections piled above, and felts irregularly developed over portions of the inner surfaces of the sections which had been ripped.

Beneath the felts on the longitudinal surfaces of sections 2 and 3 the wood showed the typical yellow-brown color of *P. cocos* rot; where these felts had overgrown the original core, the reddish color had disappeared with the development of *P. cocos* rot. The side pieces of these sections were ripped and it was found that *P. cocos* had penetrated deeply; inocula from the newly exposed wood of both sections yielded *P. cocos*.

Around the base of section 4 in contact with the floor, sporophores similar to those found on *log No. 40* had developed. These also were sterile, but cultures grown from their tissue produced sporulating surfaces.

P. cocos rot was not present in *log No. 1970* when the wood was examined in March 1953 after two years' storage in the open; it must have entered the log during the summer of 1953 after it had been cut and piled in the shed. Since the fruit bodies were in good condition when found in January, it is probable that they developed late in the season; otherwise it is to be expected that they would have been destroyed.

Felts and Sporophores

The mycelial sheets of *P. cocos* were loosely formed or closely felted, varying with the conditions under which they developed; they varied also in color from the creamy white, through pinkish shades, to dark chocolate brown. Felts of the more delicate texture and color were found in confined spaces, while leathery, chocolate brown felts developed on more exposed surfaces.

The fruit bodies were grayish white when found, but may have been a purer white when newly developed. A marginal zone at the top was smooth, grayish, and tough in texture. Pores in the upper part of the fruit bodies were whitish, those toward the bottom brown; all were large, angular, and somewhat lacerated.

References

1. DAVIDSON, R. W., CAMPBELL, W. A., and VAUGHN, D. B. Fungi causing decay of living oaks in the Eastern United States, and their cultural identification. U.S. Dept. Agr. Tech. Bull. 785. 1942.
2. REDMOND, D. R. and ROBINSON, R. C. *Poria cocos* in the Maritimes. Bi-monthly Progr. Rep., Can. Dept. Agr. Sept.—Oct. 1952.
3. WEBER, G. F. The occurrence of tuckahoes and *Poria cocos* in Florida. Mycologia, 21 : 113–130. 1929.
4. WOLF, F. A. The fruiting stage of the tuckahoe, *Pachyma cocos*. Elisha Mitchell Sci. Soc. 38 : 127. 1922.

PLATE I

FIG. 1. Sporophores of *Poria cocos* (Schw.) Wolf around base of pulpwood bolt of *Populus tremuloides* Michx.

FIG. 2. Enlargement of portion of sporophore shown in Fig. 1.

COMMONWEALTH BUREAU OF HORTICULTURE AND PLANTATION CROPS

EAST MALLING, KENT

The publications issued by this Branch of the Commonwealth Agricultural Bureaux include the following periodical and occasional publications:

HORTICULTURAL ABSTRACTS

A quarterly digest of world literature on research into the cultivation of horticultural and plantation crops including deciduous fruits, vegetables and ornamentals, drug plants, and tobacco.

Annual subscription 50*s*. through a bookseller, or, within the Commonwealth, 40*s*. by direct subscription.

TECHNICAL COMMUNICATIONS

22. E. J. Hewitt. Sand and water culture methods used in the study of plant nutrition. 1952, pp. 241, bibl. 943, illus. 42*s*. "Here is a timely book which should be in the hands of all workers in the field of plant nutrition." Agric. Inst. Rev., 1953, Vol. 8, No. 5.

23. S. C. Pearce. Field experimentation with fruit trees and other perennial plants. 1953, pp. 141, bibl. 194. 10*s*. "It should be studied section by section by all engaged in experimental work on perennial crops, for it is full of wise advice." J. hort. Sci., 1953, Vol. 28, Pt. 4.

Subscriptions and enquiries to C.A.B., Farnham Royal, Slough, Bucks, England.

Free specimen copies of Horticultural Abstracts from Bureau, East Malling.

Notes to Contributors

Manuscripts

(i) ***General***

Manuscripts should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables (each typed on a separate sheet) and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts should be carefully checked before they are submitted; authors will be charged for changes made in the proof that are considered excessive.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard.

As many photographs as possible should be mounted together (with a **very** small space between each photo) to reduce the number of cuts required. Full use of the space available should be made and the ratio of height to width should correspond to that of a journal page; however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. × 11 in.) and making allowance for illustrations (not inserts). The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents

Page

Detection of Vitamin B_{12} Production by Microorganisms Using *Euglena gracilis* var. *bacillaris* as the Assay Organism—*E. R. Witkus, F. Gentile, L. Kelly, and L. Murphy* - - - - - - - - - - - - 503

Selfing Studies with Physiologic Races of Wheat Stem Rust, *Puccinia graminis* var. *tritici*—*T. Johnson* - - - - - - - - - - 506

Phosphorylase in the Chloroplasts of Wheat—*Michael Shaw* - - - - 523

An Anatomical Study of Cane and Crown Galls—*W. E. McKeen* - - - 527

Cytological Studies in Alfalfa Polyploids—*J. M. Armstrong* - - - - 531

A Hitherto Unreported Ascomycete Parasitic on the Roots of Wheat, Oats, and Barley—*D. B. Robinson and G. W. Ayers* - - - - - - - - 543

Fructification of *Poria cocos* (Schw.) Wolf on Poplar—*Clara W. Fritz* - - 545